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(54) **GENETICALLY MODIFIED
LACTATE-CONSUMING YEASTS AND
FERMENTATION PROCESSES USING SUCH
GENETICALLY MODIFIED YEASTS**

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C12N 9/34 (2006.01)

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C12N 9/1029; **C12Y 402/01**; **C12Y**
201/01; **C12Y 114/11022**; **C12P 7/24**;
C12P 19/14; **C12P 7/00**
USPC **435/254.2**, **254.21**, **190**, **139**, **161**, **157**,
435/26

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to genetically modified yeasts that can use lactate as a carbon source to produce a fermentation product. In one aspect, the yeasts can consume gluconse and lactate simultaneously to produce ethanol. In one aspect, the genetically modified yeast is transformed to include a monocarboxylic/monocarboxylate transporter. In one aspect, the yeast can include one or more heterologous genes encoding lactate dehydrogenase (cytochrome) (EC 1.1.2.3 and/or 1.1.2.4).

9 Claims, 9 Drawing Sheets

Specification includes a Sequence Listing.

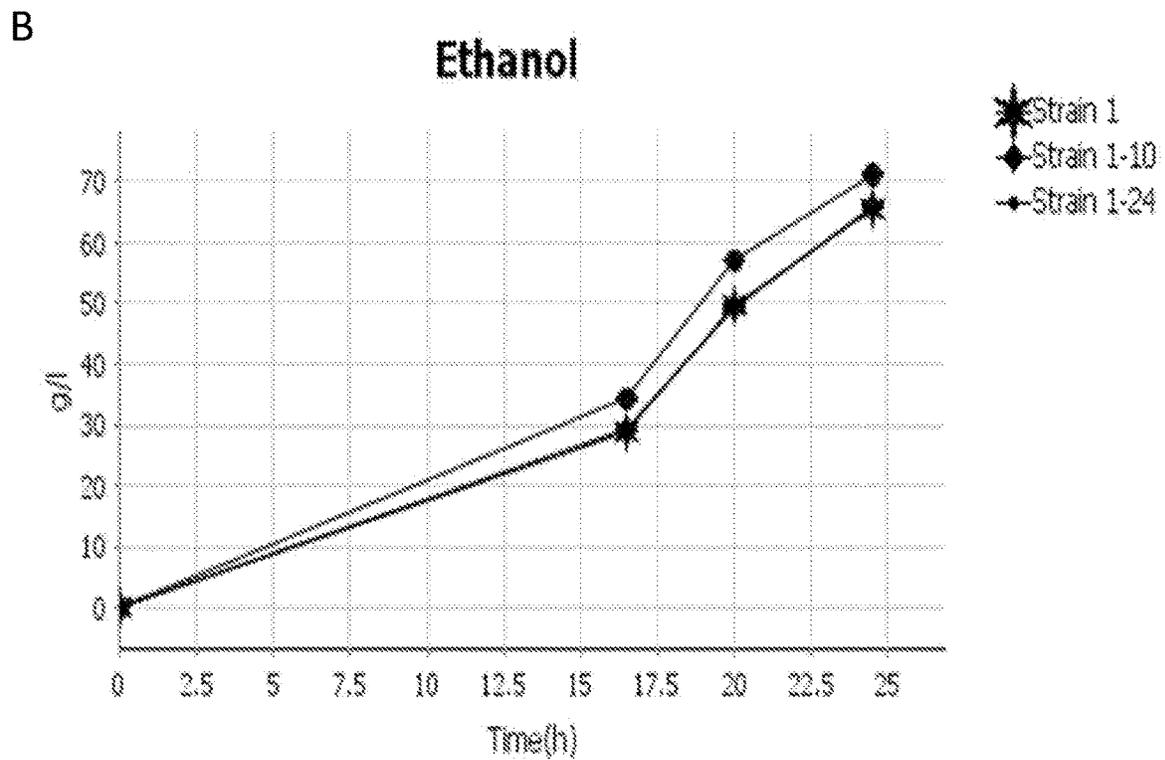
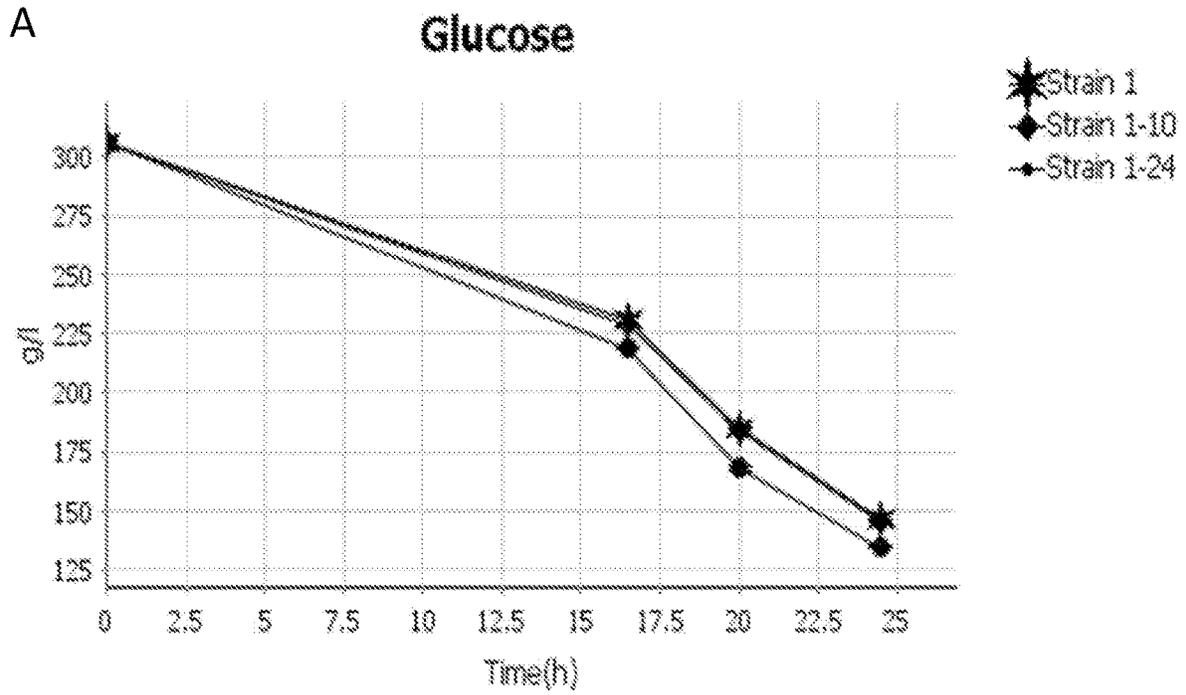


Figure 1A-1B

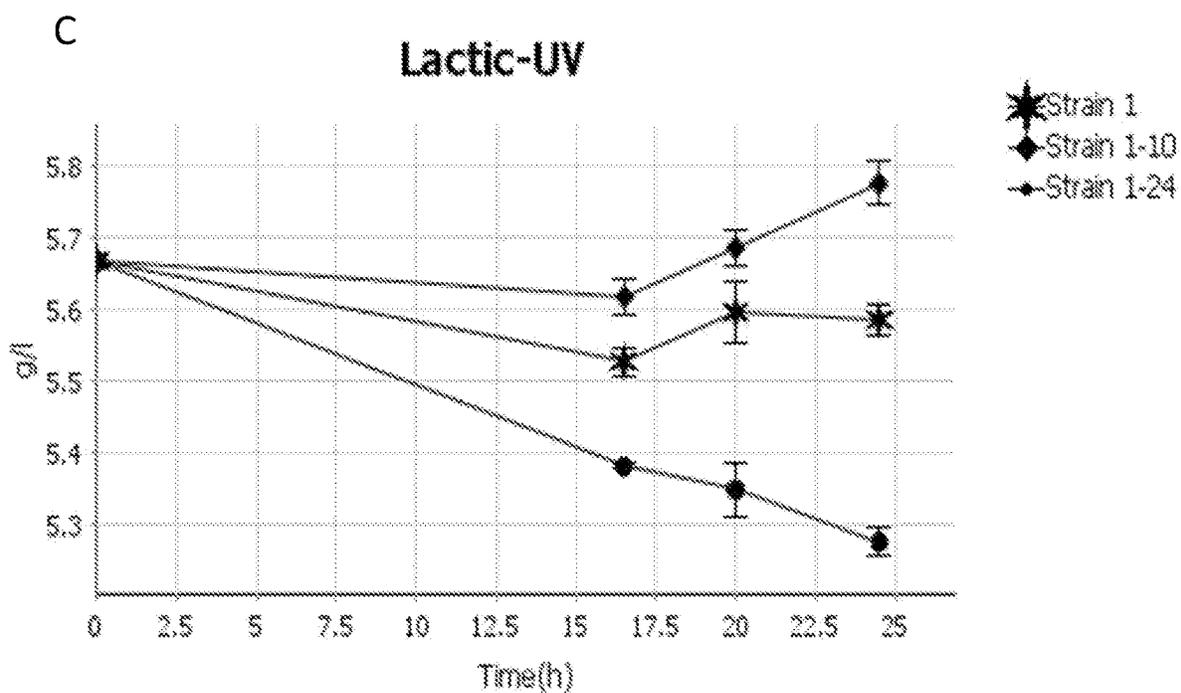


Figure 1C

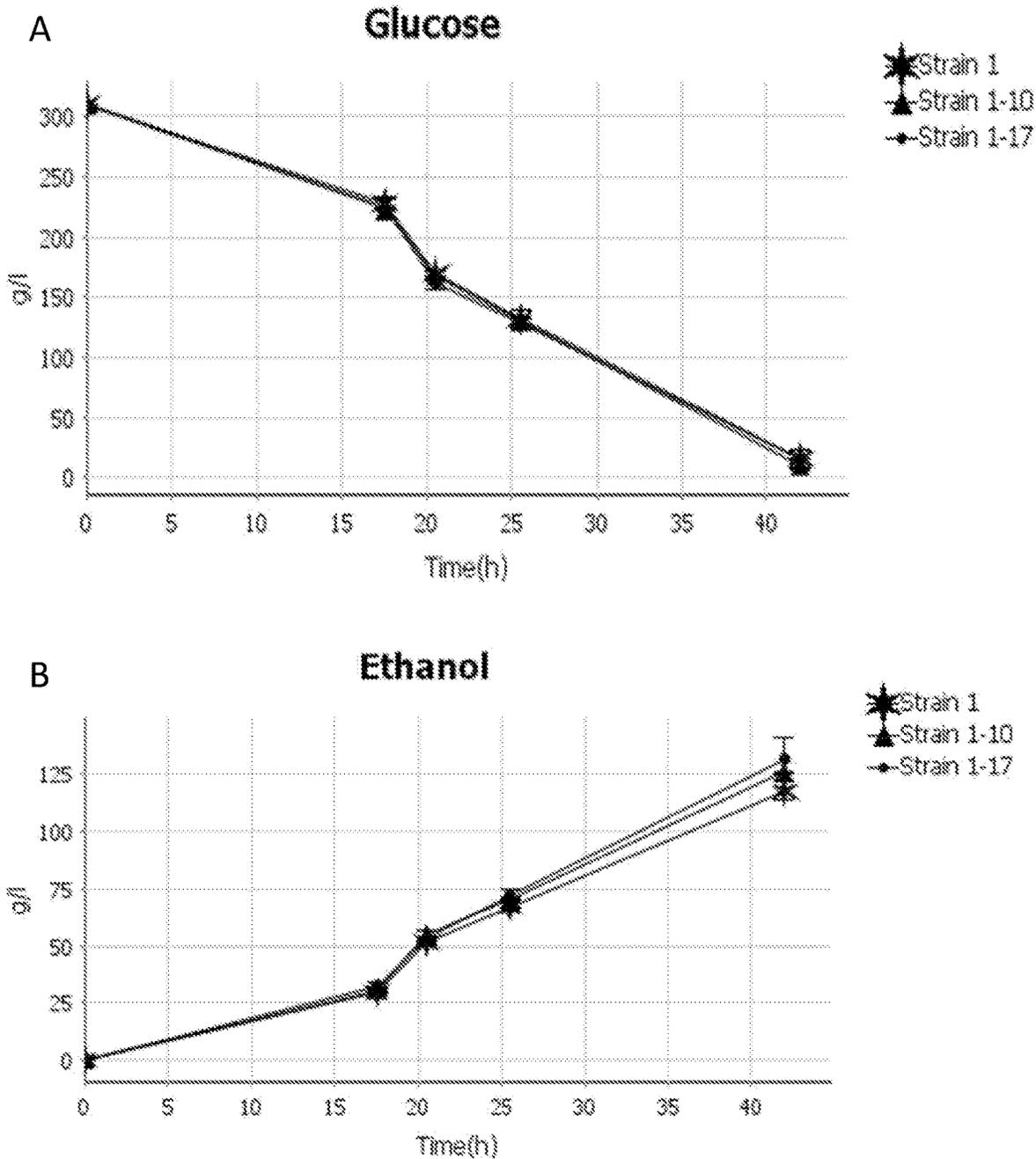


Figure 2A-2B

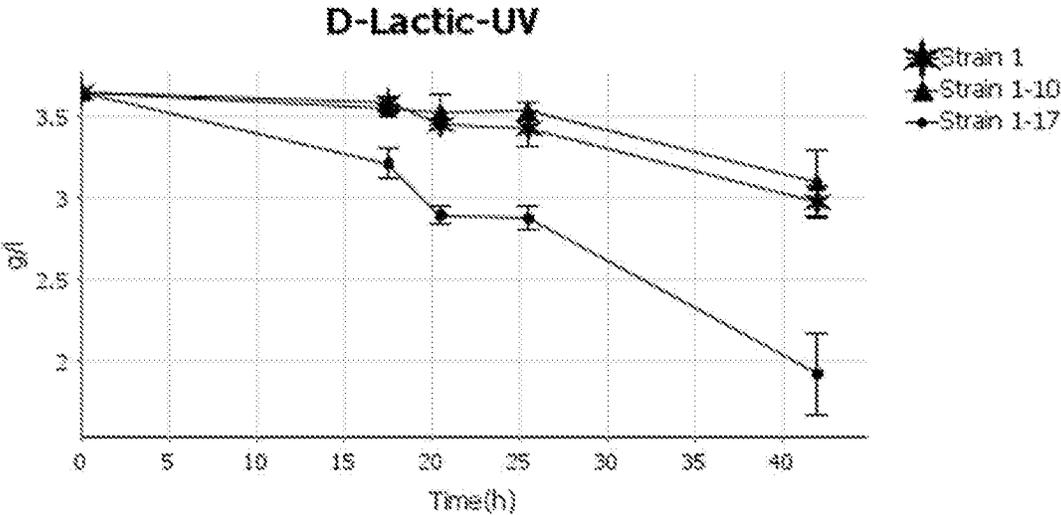


Figure 2C

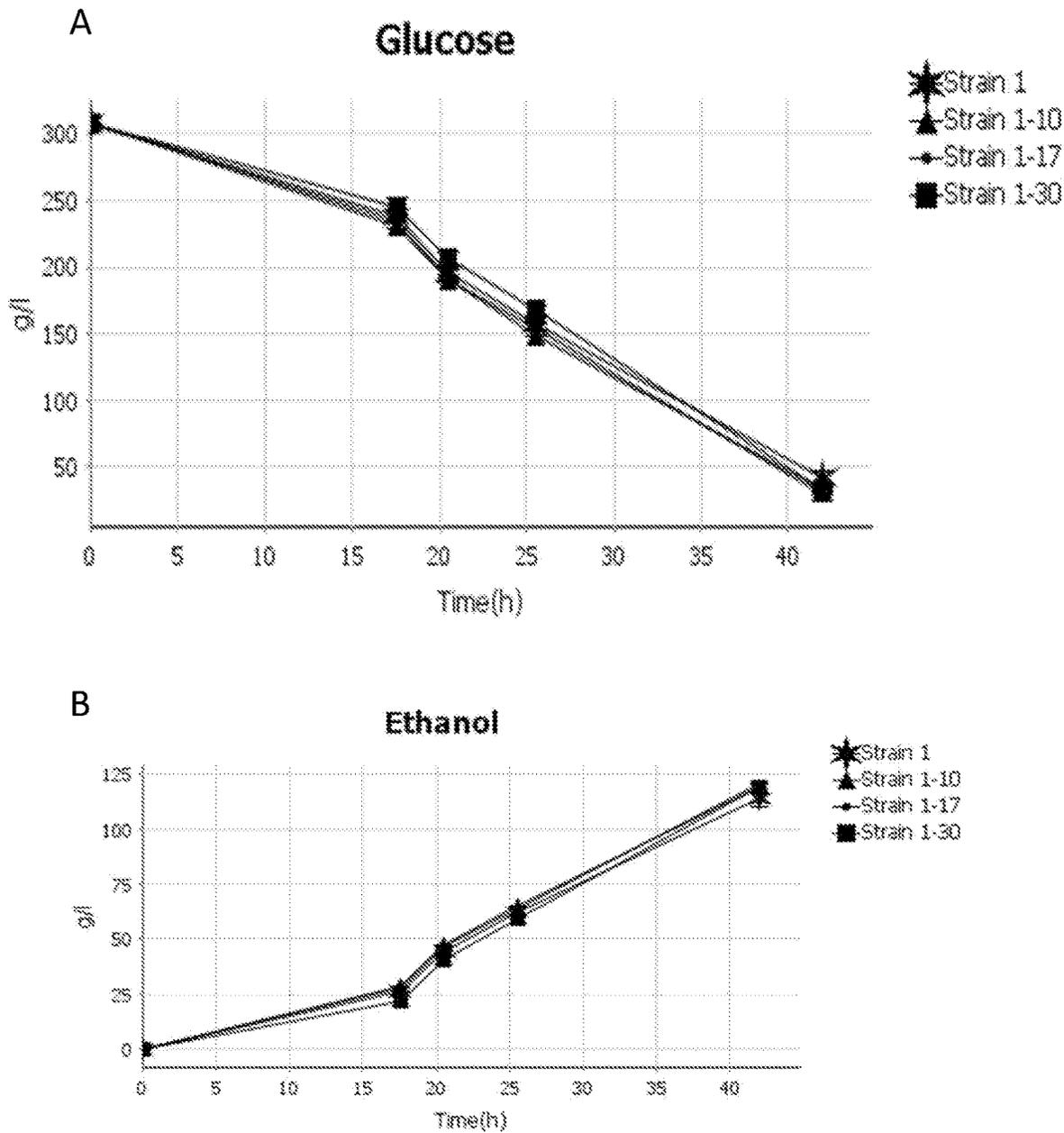


Figure 3A-3B

C

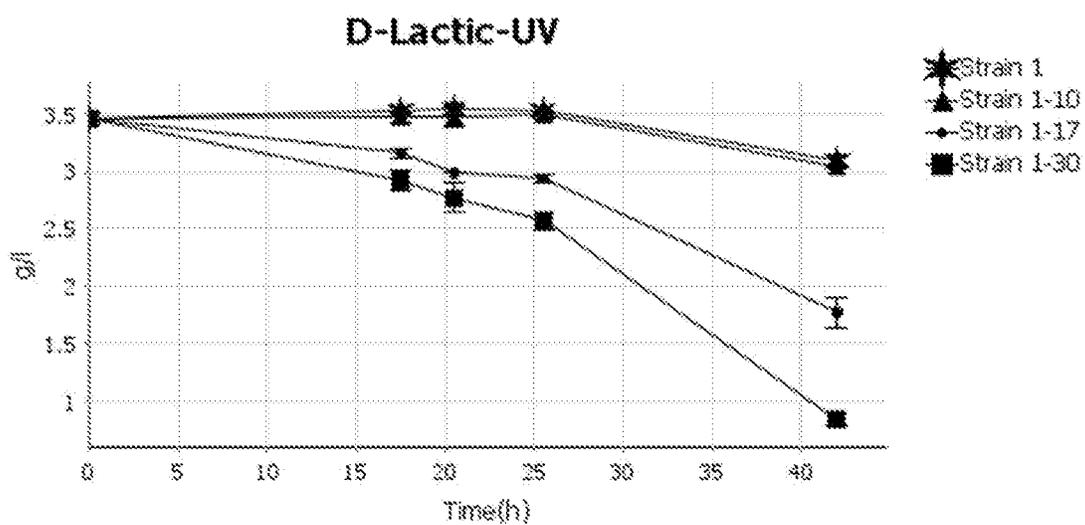
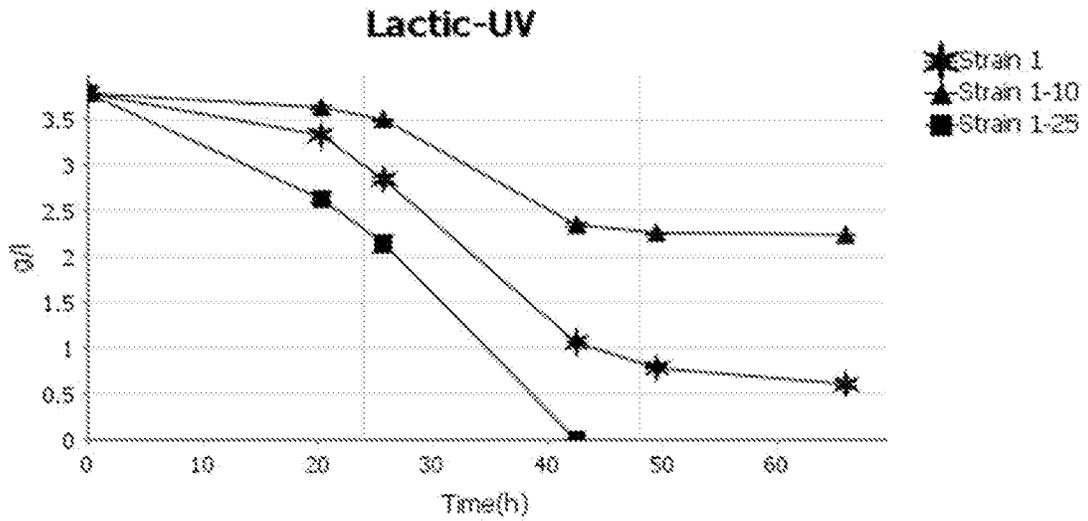


Figure 3C

A



B

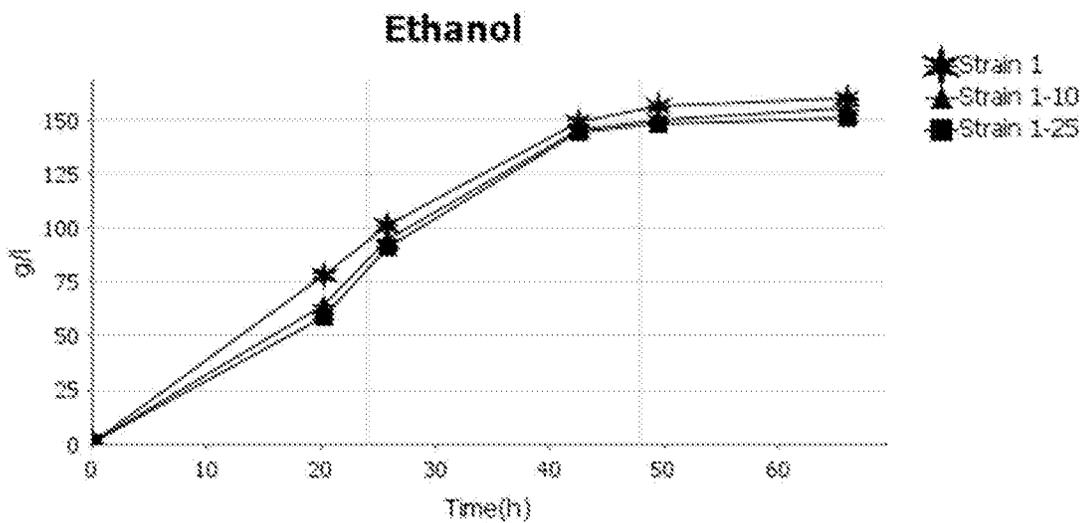
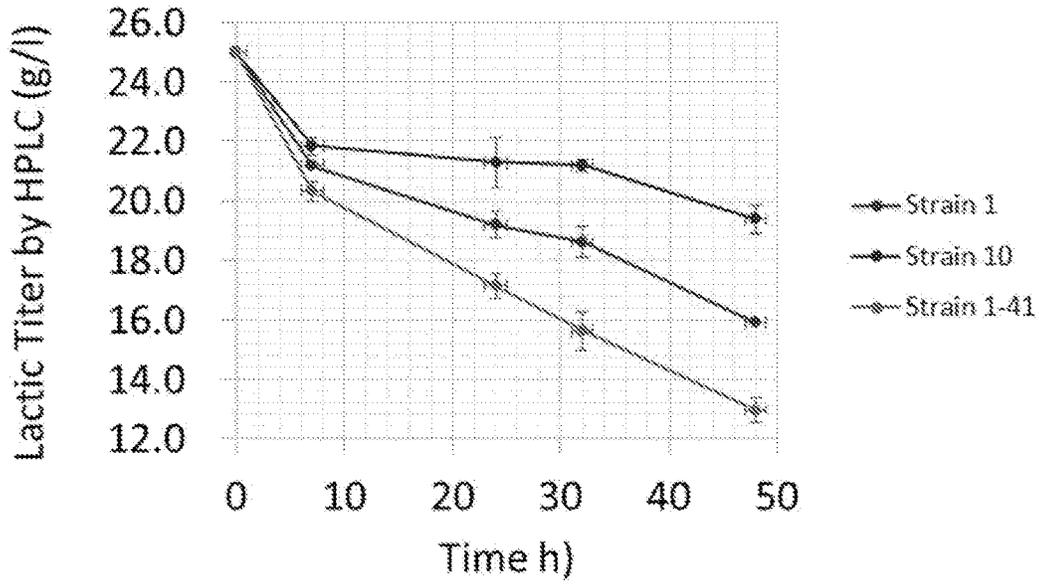


Figure 4

A



B

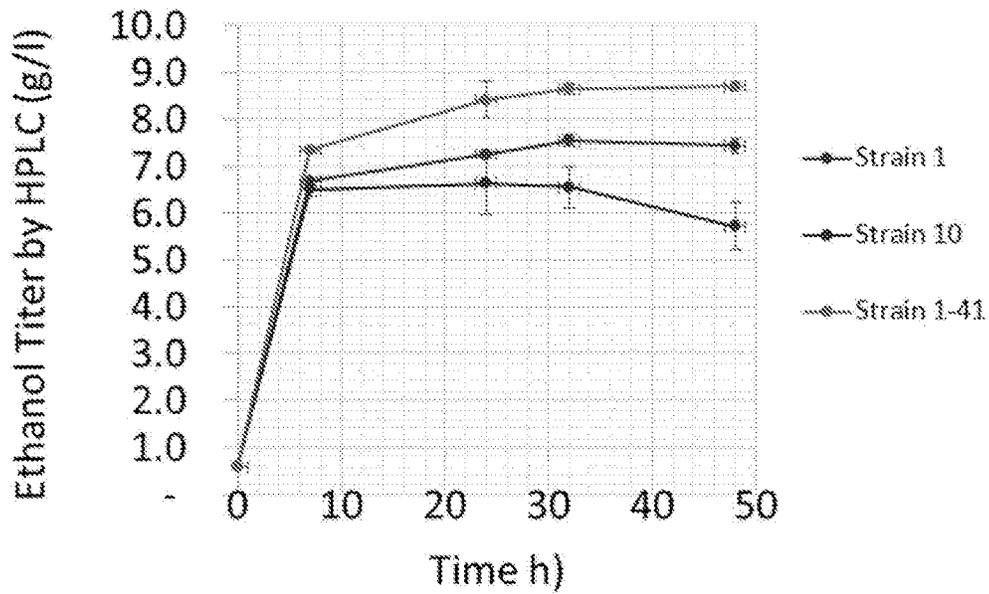


Figure 5

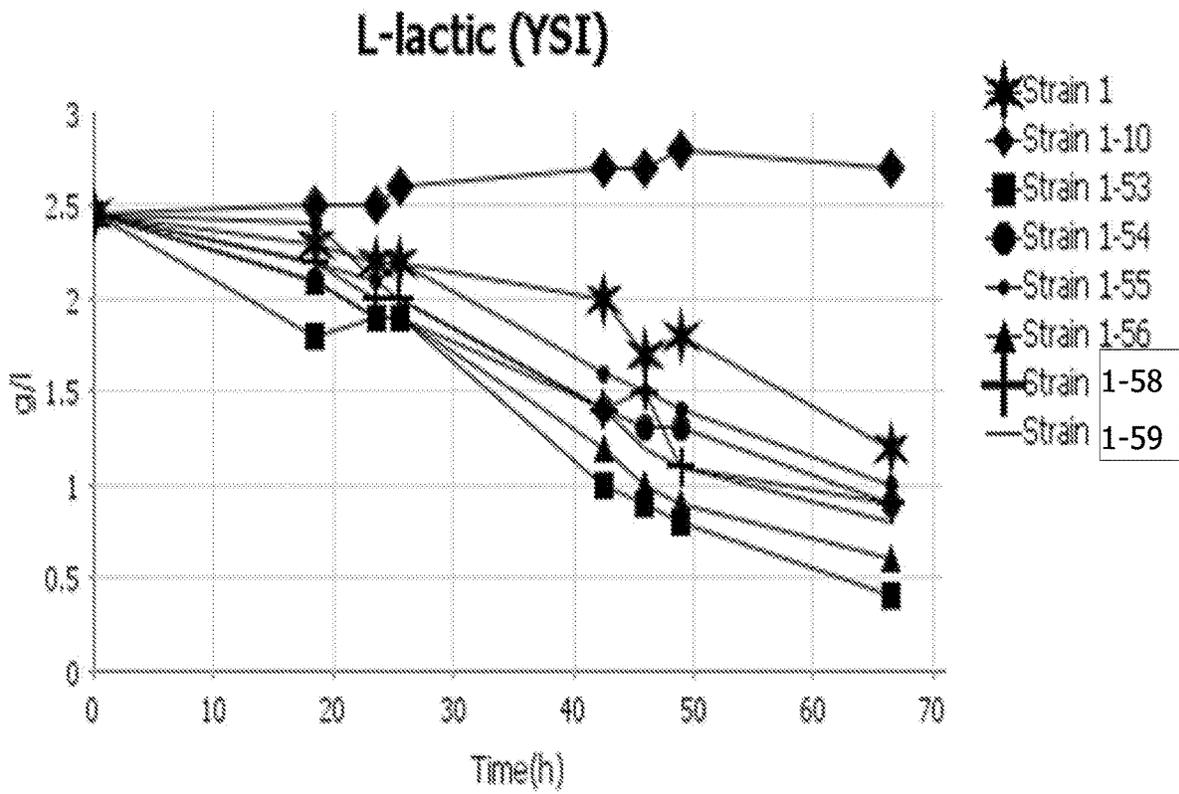


Figure 6

**GENETICALLY MODIFIED
LACTATE-CONSUMING YEASTS AND
FERMENTATION PROCESSES USING SUCH
GENETICALLY MODIFIED YEASTS**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a national phase application of PCT/US2017/051720, filed Sep. 15, 2017, entitled GENETICALLY MODIFIED LACTATE-CONSUMING YEASTS AND FERMENTATION PROCESSES USING SUCH GENETICALLY MODIFIED YEASTS, which claims the benefit of U.S. Provisional Patent Application No. 62/395,792, filed Sep. 16, 2016, entitled GENETICALLY MODIFIED LACTATE-CONSUMING YEASTS AND FERMENTATION PROCESSES USING SUCH GENETICALLY MODIFIED YEASTS, each of which is hereby incorporated by reference herein in its entirety.

SEQUENCE LISTING

The entire contents of the ASCII text file entitled "N00478_ST25.txt," created on Sep. 15, 2017, and having a size of 506 kilobytes, is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Industrial yeast fermentation processes often use corn process streams, for example corn steepwater, as a fermentation substrate. These process streams contain starches that can be converted to sugars via treatment with saccharification enzymes. In current fermentation processes, gluconase or other sugars serve as the carbon source for fermentation. However, such process streams can contain a significant amount of lactate which cannot be efficiently used as a carbon source by yeasts, especially in the presence of gluconase.

SUMMARY OF THE INVENTION

Described herein are genetically modified yeasts that can consume lactate during fermentation. In one aspect, the yeasts can use lactate as a carbon source to form a fermentation product. In one aspect, the yeasts can consume lactate and gluconase simultaneously during a fermentation process to produce a fermentation product. In some embodiments, the fermentation product is ethanol (EtOH). In some embodiments, the yeast is a genetically modified *Saccharomyces cerevisiae* yeast. In some embodiments, the yeast is transformed to express a heterologous monocarboxylate/proton symporter, e.g., a JEN1 symporter. In some embodiments, the yeast is transformed to express one or more heterologous genes encoding a lactate dehydrogenase (cytochrome) (EC 1.1.2.3 or 1.1.2.4).

In one aspect, the genetically modified yeast comprises a heterologous gene encoding a monocarboxylic/monocarboxylate transporter and one or more heterologous genes encoding lactate dehydrogenase (cytochrome) (classified as EC 1.1.2.3 or 1.1.2.4), wherein the yeast is capable of consuming lactate and producing ethanol when the yeast is present in a fermentation medium comprising lactate and hexose. In some embodiments, the yeast is capable of consuming D-lactate, L-lactate, or a mixture thereof. In some embodiments, the yeast is capable of consuming both D-lactate and L-lactate. In some embodiments, the yeast has

a L-lactate consumption rate of at least 0.030, 0.035, 0.040, 0.045, or 0.050 g L⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 150 g/L or greater. In some embodiments, the yeast has a L-lactate consumption rate of at least 0.015, 0.020, 0.025, 0.030, 0.035, or 0.040 g L⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 5 g/L or greater. In some embodiments, the yeast has a D-lactate consumption rate of at least 0.005, 0.006, 0.007, 0.008, 0.009, 0.010, 0.015, 0.020, 0.025, or 0.030 g L⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 150 g/L or greater. In some embodiments, the yeast has a D-lactate consumption rate of at least 0.015, 0.020, 0.025, 0.030, 0.035, or 0.040 g L⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 5 g/L or greater. In some embodiments, the lactate consumption rate of D-lactate, L-lactate, or total lactate for the yeast is determined according to the "Evaluation Protocol for Lactate Consumption" described later herein.

In some embodiments, the yeast has a total lactate consumption rate (i.e., consumption of both D- and L-lactate) of at least 0.550, 0.555, 0.600 or 0.650 g L⁻¹h⁻¹ between time 0 and 7 hours in a simultaneous saccharification fermentation (SSF) process. In some embodiments, the yeast has a total lactate consumption rate of at least 0.130, 0.140, 0.150, 0.160, or 0.170 g L⁻¹h⁻¹ between time 7 hours and 48 hours in a SSF process. In some embodiments, the yeast has a total lactate consumption rate of at least 0.200, 0.210, 0.220, 0.230, 0.240, or 0.250 g L⁻¹h⁻¹ between time 0 and 48 hours in a SSF process. In some embodiments, the total lactate consumption rate is determined according to the method for SSF shake flask assay in Example 4 described later herein.

In one aspect, the one or more heterologous lactate dehydrogenase (cytochrome) genes comprise an overexpressed D-lactate dehydrogenase (DLD) gene (EC 1.1.2.4). In one aspect, the one or more heterologous lactate dehydrogenase (cytochrome) genes comprise an overexpressed cytochrome b2 (CYB2) gene (EC 1.1.2.3). In some embodiments, the one or more heterologous lactate dehydrogenase (cytochrome) genes comprise an overexpressed DLD gene and an overexpressed CYB2 gene. In some embodiments, the one or more DLD genes is from the yeast genus of *Saccharomyces*, *Issatchenkia*, and/or *Kluyveromyces*. In some embodiments, the one or more DLD genes encodes for a DLD polypeptide having an amino acid sequence with a sequence identity of at least 90 or 95% to the DLD polypeptide from one or more of the following yeast species: *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, *Saccharomyces kluyveri*, *Saccharomyces bayanus*, *Kluyveromyces dobzhanskii*, *Kluyveromyces marxianus*, or *Kluyveromyces lactis*. In some embodiments, the yeast encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequences: SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, or SEQ ID NO: 74.

In some embodiments, the one or more CYB2 genes is from the yeast genus of *Saccharomyces*, *Issatchenkia*, *Zygosaccharomyces*, and/or *Kluyveromyces*. In some embodiments, the one or more CYB2 genes encodes for a CYB2 polypeptide having an amino acid sequence with a sequence identity of at least 90 or 95% to the CYB2 polypeptide from one or more of the following yeast species: *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, *Saccharomyces kluyveri*, *Saccharomyces bayanus*, *Zygosaccharomyces rouxii*, *Kluyveromyces dobzhanskii*, or *Kluyveromyces lactis*. In some embodiments, the yeast encodes for a polypep-

tide having an amino acid sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequences: SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62.

In one aspect, the yeast comprises heterologous genes other than a DLD, a CYB2, or a heterologous monocarboxylate/proton symporter. In some embodiments, the yeast comprises a heterologous gene encoding a lactate racemase.

In one aspect, the genetically modified yeast comprises a heterologous gene encoding a monocarboxylate/proton symporter (JEN1) and a heterologous gene encoding glucoamylase, wherein the yeast is capable of consuming lactate and producing ethanol when the yeast is present in a fermentation medium comprising lactate, and further comprising hexose, starch, or a combination thereof. In one aspect, any of the above yeasts be transformed with a heterologous glucoamylase gene. In some embodiments, the yeast can encode for a glucoamylase from *Saccharomycopsis fibuligera* or *Rhizopus oryzae*. In some embodiments, the yeast encodes for a glucoamylase polypeptide having at least 80%, 85%, 90%, or 95% sequence identity to the wild type glucoamylase polypeptide from *Saccharomycopsis fibuligera*. Embodiments of glucoamylase polypeptides that can be encoded by the yeast, and also the DNA sequences encoding such peptides that can be integrated in the yeast, are further described in International Patent Application No. PCT/US17/045493, filed 4 Aug. 2017, which is hereby incorporated by reference in its entirety, including all SEQ IDs associated therewith.

In one aspect, the host yeast that is genetically modified is of the species *Saccharomyces*. In some embodiments, the host yeast is *Saccharomyces cerevisiae*. In some embodiments, the genetically modified yeast comprises a *Saccharomyces cerevisiae* yeast, wherein the yeast comprises a heterologous gene encoding a monocarboxylate/proton symporter (e.g., JEN1). In some embodiments, the host yeast is a *Saccharomyces cerevisiae* yeast that is commercially available for use in ethanol production, such as ETHANOL RED™ yeast, which at the time of this disclosure is available from Lesaffre or a subsidiary thereof.

In one aspect, the yeast is capable of producing ethanol at a fermentation production rate of at least 1.0 g L⁻¹ h⁻¹, 2.0 g L⁻¹ h⁻¹, 3.0 g L⁻¹ h⁻¹, 3.3 g L⁻¹ h⁻¹, or 3.75 g L⁻¹ h⁻¹. In one aspect, the yeast is capable of producing ethanol at a titer greater than 80 g/L, 90 g/L, 100 g/L, 110 g/L, 120 g/L, 125 g/L, 130 g/L, 135 g/L, 140 g/L, 145 g/L, 150 g/L, 155 g/L, 160 g/L, or 165 g/L. In one aspect, the yeast is (a) capable of producing ethanol at a titer of greater than 90 g/L, 100 g/L, 110 g/L, 120 g/L, 130 g/L, or 140 g/L; (b) thermotolerant at temperatures in the range of 33° C. to 40° C., 33° C. to 39° C., 33° C. to 38° C., 33° C. to 37° C., 34° C. to 37° C., 35° C. to 37° C., or 36° C. to 38° C.; or both (a) and (b).

In one aspect, the heterologous gene encoding a monocarboxylic/monocarboxylate transporter is from a yeast of the genus *Kluyveromyces*. In some embodiments, the heterologous gene encoding a monocarboxylic/monocarboxylate transporter is from *Kluyveromyces lactis*. In some embodiments, the yeast encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequences: SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, or SEQ ID NO: 78. In some embodiments, the yeast comprises one or more of the following residues at the indicated positions in SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ

ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62: Lys349, Tyr143, Tyr254, and His373. In one aspect, yeast is used for the production of ethanol or a bioproduct other than ethanol.

In one aspect, the present disclosure relates to fermentation processes using a genetically modified yeast, for example any of the genetically modified yeasts described above. In some embodiments, the process is any process for fermenting a medium to produce ethanol using such genetically modified yeasts. In some embodiments, the process is a continuous fermentation process for manufacturing ethanol comprising: providing a fermentation medium comprising hexose or hexose oligomers, fermenting the fermentation medium with a genetically modified yeast comprising a heterologous monocarboxylate/proton symporter (JEN1) gene, adding one or more feed streams comprising lactate to the fermentation medium, and removing at least one output stream comprising ethanol from the fermentation medium, wherein the average hexose or hexose oligomer concentration of the fermentation medium is at least 5 g/L, the volumetric oxygen uptake rate of the process is at least 0.5 mmol O₂/(L·h), and the process produces ethanol at an overall rate of at least 1.0 g L⁻¹ h⁻¹, and the yeast consumes lactate, wherein the at least one output stream contains less than 90% of the lactate added in the one or more feed streams. In some embodiments, the lactate concentration of one or more feed streams has an average lactate concentration of at least 1 g/L. In some embodiments, the feed stream is a vegetable process stream. In some embodiments, the vegetable process stream is a corn process stream or a wheat process stream. In some embodiments, the ethanol titer at the end of the pre-fermenter step is in the range of 20 to 80 g/L. In some embodiments, the lactate consumption rate in the propagator step and/or pre-fermenter step is at least 0.550, 0.600, or 0.650 g L⁻¹ h⁻¹. In some embodiments, the ethanol titer of the at least one output stream is at least 90, 100, 110, 120, 130, 140, 150, or 160 g/L.

In some embodiments, the process is a process for manufacturing ethanol comprising: fermenting a medium using a genetically modified yeast comprising a heterologous monocarboxylate/proton symporter (JEN1) gene, wherein the medium comprises gluconse or gluconse oligomers at a concentration of at least 5 g/L at the start of fermentation, the lactate content of the medium is at least 1 g/L at the start of fermentation, at least 35, 40, 45, 50% or more of the lactate is consumed at the end of fermentation, and the final ethanol titer is at least 90 g/L. In some embodiments, the process has a volumetric oxygen uptake rate (OUR) is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or 100 mmol O₂/(L·h).

In one aspect, the lactate can be L-lactate, D-lactate, or a mixture thereof. In some embodiments, the pH of the fermentation medium is in the range of about 2 to 7. In some embodiments, the fermentation temperature is in the range of 25 to 45° C., 25 to 40° C., 25 to 35° C., 25 to 30° C., 30 to 45° C., 30 to 40° C., 25 to 38° C., 28 to 38° C., or 30 to 39° C. In some embodiments, the final ethanol titer of the process is at least 80, 90, 100, 110, 120, 130, 135, 140, 145, 150, 155, or 160 g/liter.

In some embodiments, the dry solids of the fermentation medium is at least 30, 40, 50, 60, 70, or 80 g/L in the fermentation medium. In some embodiments, the dry solids of the fermentation feed is at least 120, 130, 140, or 150 g/L.

In one aspect, the process is a process for manufacturing ethanol comprising: fermenting a medium using a *Saccharomyces cerevisiae* yeast, for example Ethanol Red™ yeast (commercially available from Lesaffre), wherein the medium comprises gluconse or gluconse oligomers and

lactate, the OUR is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mmol O₂/(L·h), the lactate content of the medium is at least 1, 2, 3 or 4 g/L at the start of fermentation, at least 35, 40, 45, or 50% of the lactate is consumed at the end of fermentation, and the final ethanol titer is at least 90 g/L.

In one aspect, the method is a method for producing a genetically modified yeast with improved lactate consumption comprising overexpressing one or more heterologous genes encoding a lactate dehydrogenase (cytochrome) (classified as EC 1.1.2.3 or 1.1.2.4) or a heterologous monocarboxylate/proton symporter, and subjecting the yeast to evolution for a characteristic, wherein the yeast is capable of consuming lactate and producing ethanol when the yeast is present in a fermentation medium comprising lactate and gluconse. In some embodiments, the characteristic is increased growth rate of the yeast on lactate and/or increased ethanol production of the yeast in the presence of lactate.

In one aspect, in any of the above methods or processes or yeasts, the yeast encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequence: SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, or SEQ ID NO: 78. In some embodiments, the yeast comprises one or more of the following residues at the indicated positions in SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62: Lys349, Tyr143, Tyr254, and His373.

In one aspect, in any of the above methods or processes or yeasts, the yeast includes a heterologous nucleic acid regulatory sequence associated with the heterologous gene encoding a monocarboxylic/monocarboxylate transporter. In some embodiments, the heterologous nucleic acid regulatory sequence comprises the ADH1 promoter. In some embodiments, the heterologous nucleic acid regulatory sequence comprises the ADH2 promoter, the PDC1 promoter, or the GPD1 promoter.

In one aspect, the genetically modified yeasts are suitable for ethanol production at a commercially acceptable performance. Accordingly, in any of the above embodiments, the yeast and processes using the yeast are associated with commercially relevant final ethanol titers, ethanol titers at the end of fermentation, or process streams or materials removed from the process having ethanol concentrations of at least 90, 100, 110, 120, 130, 140, 150, or 160 g/L. Further, in some embodiments, any of the above yeasts has a D-, L-, and/or total lactate consumption rate that is greater than a yeast without a heterologous gene encoding a monocarboxylic/monocarboxylate transporter.

In one aspect, any of the genetically modified yeasts comprising a heterologous gene encoding a monocarboxylic/monocarboxylate transporter described above or elsewhere herein are described as capable of consuming lactate and producing ethanol while gluconse is present, i.e., while in a fermentation medium comprising gluconse. In some embodiments, the fermentation medium comprises a measurable, non-zero concentration of gluconse. However, it is contemplated herein that in some embodiments, the gluconse concentration of the fermentation medium may not be practically measurable because the gluconse may be added to the fermentation medium or otherwise generated within the fermentation medium from gluconse oligomers in such a manner that the yeast present in the fermentation medium will consume the gluconse immediately. Therefore, it is to be understood that a "fermentation medium comprising gluconse" can include a fermentation medium wherein the

gluconse is not practically measurable due to the gluconse being consumed by the yeast as soon as it is generated or added to the fermentation medium.

It is also to be understood that the elements or aspects of any embodiment of the processes, methods, or compositions described above can be applied to any other embodiment, as would be understood by a person skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of the invention will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

FIG. 1, comprising FIGS. 1A through 1C, is a set of graphs showing gluconse consumption, ethanol production, and L-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* reference strains (1 and 1-10) and a strain containing an overexpressed CYB2 and JEN1 (1-24).

FIG. 2, comprising FIGS. 2A through 2C, is a set of graphs showing gluconse consumption, ethanol production, and D-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* reference strains (1 and 1-10) and a strain containing an overexpressed *Kluyveromyces lactis* JEN1 (1-17).

FIG. 3, comprising FIGS. 3A through 3C, is a set of graphs showing gluconse consumption, ethanol production, and D-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* reference strains (1 and 1-10), a strain containing an overexpressed *K. lactis* JEN1 (1-17) and a strain containing an overexpressed *K. lactis* JEN1 and an overexpressed ScDLD1 (1-30).

FIG. 4, comprising FIGS. 4A and 4B, is a set of graphs showing D,L-lactate consumption and ethanol production in simultaneous saccharification fermentation (SSF) shake flask assays for *Saccharomyces cerevisiae* reference strains (1 and 1-10) and a strain containing an overexpressed CYB2b and a JEN1 (1-25).

FIG. 5, comprising FIGS. 5A and 5B, is a set of graphs showing D,L-lactate consumption and ethanol production in SSF shake flask assays for *Saccharomyces cerevisiae* reference strains (1 and 1-10) and a strain containing two copies of both an overexpressed ScCYB2 and a K1JEN1 (1-41).

FIG. 6 is a graph showing L-lactate consumption in shake flask assays for *Saccharomyces cerevisiae* reference strains (1 and 1-10) and strains containing an overexpressed *S. cerevisiae* DLD1 and *S. cerevisiae* CYB2, and selected monocarboxylate/proton symporters.

DETAILED DESCRIPTION

It is to be understood that the figures and descriptions of the present invention provided herein have been simplified to illustrate elements that are relevant for a clear understanding of the present invention, while eliminating other elements found in the related field(s) of art. Those of ordinary skill in the art would recognize that other elements or steps may be desirable or required in implementing the present invention. However, because such elements or steps are well known in the art or do not facilitate a better understanding of the present invention, a discussion of such elements or steps is not provided herein.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one skilled in the art to which this invention belongs. As used herein, each of the following terms has the meaning associated with it as defined in this section.

As used herein, the terms “genetically modified,” “genetically engineered,” and the like refer to the altering of the genetic material of an organism in any way using any genetic engineering technique, including techniques using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins, i.e., CRISPR/cas systems.

As used herein, “inoculation” is defined as the point in time wherein a microorganism capable of producing a fermentation product is introduced into a fermentation medium.

The terms “bioproduct,” “fermentation product,” and the like are used interchangeably herein and refer to a compound or mixture of compounds produced by an organism via fermentation of a carbon source.

As used herein, “end of fermentation” is defined as the point in time where a fermentation process meets a predetermined endpoint criteria. The predetermined endpoint criteria can include any of the following: a predetermined time interval, exhaustion of the desired fraction of carbon source supplied, cessation of carbon source consumption, or cessation of fermentation product formation. In one embodiment, “end of fermentation” is defined as the point in time where harvesting of the bioproduct is started. As would be understood by a person skilled in the art, “end of fermentation” can refer to a point in time that is different depending on the scale and purpose of the fermentation process. For a large-scale production fermentation process, the “end of fermentation” is preferably the point at which harvesting of the bioproduct is started, i.e., after product formation has effectively stopped. As would be understood by a person skilled in the art, the endpoint criteria for a batch process applies to the entire batch, whereas the endpoint criteria for a continuous process may apply to only a portion of the process, for example a single vessel or unit operation associated with the process, e.g., the process step associated with the pre-fermenter, or may apply to the entire continuous process, i.e., to an output parameter of the continuous process.

As used herein, “cell dry weight” refers to the concentration of dry cell mass present in a fermentation medium at the time of measurement, as measured in a fermentation sample. Cell dry weight is commonly expressed in units of grams/liter (g/L).

As used herein, “cell dry weight at inoculation” refers to the concentration of dry cell mass present in a fermentation medium immediately following inoculation, as measured in a fermentation sample. For fed-batch fermentations, the initial cell dry weight is calculated based on the final volume of fermentation medium. Measurement of dry cell weight is a method known to those skilled in the art. Cell dry weight at inoculation is commonly expressed in units of g/L.

As used herein, “cell dry weight at end of fermentation” refers to the concentration of dry cell mass present in a fermentation medium at the end of fermentation, as measured in a fermentation sample. Cell dry weight at end of fermentation is commonly expressed in units of g/L.

As used herein, “final titer” refers to the concentration of a substance in the fermentation broth at the end of fermentation. The final titer is commonly expressed in units of g/L.

As used herein, “initial titer” refers to the concentration of a substance present at inoculation. The initial titer is commonly expressed in units of g/L.

As used herein, “batch time” refers to the amount of time that has elapsed between the inoculation and the end of fermentation. The batch time is commonly expressed in units of hours (h).

As used herein, “fermentation production rate” for a batch process refers to the final titer minus initial titer of fermentation product (final titer minus initial titer) divided by the batch time. The production rate is commonly expressed in units of grams per liter-hour ($\text{g L}^{-1} \text{h}^{-1}$). When applied to a continuous or semi-continuous process, the “fermentation production rate” is determined using methods known in the art.

As used herein, the “specific production rate” refers to the fermentation production rate divided by the cell dry weight at the end of fermentation. The specific production rate is commonly expressed in units of $(\text{g product}) (\text{g cells})^{-1} \text{h}^{-1}$. When applied to a continuous or semi-continuous process, the “specific production rate” is determined using methods known in the art.

As used herein, “product yield” of a fermentation product refers to a ratio of two quantities: a) mass of product (e.g., ethanol) produced in the course of the fermentation (numerator) b) the mass of carbon source added to the fermentation (denominator). The product yield as a percentage is commonly expressed in units of gram per gram (g/g) times 100. Particular note should be taken that product yield is calculated as a ratio of masses. The mass of fermentation product produced should account for the mass of fermentation product present in the fermentation medium at the end of the batch, as well as the mass of any fermentation product harvested during the course of the batch, less the mass of fermentation product present at the start of batch, and further less the mass of any fermentation product added during the course of the batch. The mass of carbon source added to the batch should include the mass of all carbon source(s) present in the fermenter at the start of the batch in addition to the mass of any carbon source(s) added during the course of the batch.

As used herein, “oxygen uptake rate” (“OUR”) refers to the volumetric rate at which oxygen is consumed during a fermentation. Inlet and outlet oxygen concentrations can be measured with exhaust gas analysis, for instance by mass spectrometers. OUR can be calculated by one of ordinary skill in the relevant arts using the Direct Method described in *Bioreaction Engineering Principles* 2nd Edition, 2003, Kluwer Academic/Plenum Publishers, p. 449, equation 1, which is hereby incorporated by reference. Volumetric OUR is commonly measured in units of $(\text{mmol O}_2) \text{L}^{-1} \text{h}^{-1}$.

As used herein, “specific oxygen uptake rate” refers to the specific rate at which oxygen is consumed during a fermentation. It is calculated as the ratio of the OUR to the measured cell dry weight. Specific OUR is commonly measured in units of $\text{mmol O}_2 (\text{g cell dry weight})^{-1} \text{h}^{-1}$.

The term “exogenous” as used herein with regard to genetic components means that the genetic component is present in a modified version of a microorganism, but is not present in the genome of a native form of the particular microorganism cell. In some embodiments, the exogenous genetic component can be a modified form of a component that was native to the cell, it can be derived from another organism, it can be a modified form of a component derived from another organism, or it can be a synthetically-derived component. For example, the *Kluyveromyces lactis* JEN1 gene is exogenous when introduced into *S. cerevisiae*. The

term “exogenous” as used herein with regard to a molecule means the molecule originates from outside an organism being referenced, e.g., an enzyme that is exogenous to an organism present in a fermentation broth refers to an enzyme that is not produced and/or secreted by the organism present in the fermentation broth.

As used herein, the term “heterologous” refers to a molecule or activity that is from a source that is different than the referenced organism. In some embodiments, a gene that is referred to as heterologous to a referenced organism is a gene not found in the organism. In some embodiments, an activity that is referred to as heterologous to a referenced organism is an activity not typically associated with that organism. For example, a heterologous activity in a referenced organism can include an overexpression of a gene that is found in the organism. In some embodiments, heterologous can refer to the inclusion of multiple copies of a gene that is found in a referenced organism, i.e., more copies than are typically found in the referenced organism. In some embodiments, a heterologous gene can refer to the inclusion of a gene in a different locus than where the gene is found in the native version of a referenced organism.

Inspection of nucleic acid or amino acid sequences for two nucleic acids or two polypeptides will reveal sequence identity and similarities between the compared sequences.

Sequence alignment and generation of sequence identity include global alignments and local alignments which are carried out using computational approaches. An alignment can be performed using BLAST (National Center for Biological Information (NCBI) Basic Local Alignment Search Tool) version 2.2.31 software with default parameters Amino acid % sequence identity between amino acid sequences can be determined using standard protein BLAST with the following default parameters: Max target sequences: 100; Short queries: Automatically adjust parameters for short input sequences; Expect threshold: 10; Word size: 6; Max matches in a query range: 0; Matrix: BLOSUM62; Gap Costs: (Existence: 11, Extension: 1); Compositional adjustments: Conditional compositional score matrix adjustment; Filter: none selected; Mask: none selected. Nucleic acid % sequence identity between nucleic acid sequences can be determined using standard nucleotide BLAST with the following default parameters: Max target sequences: 100; Short queries: Automatically adjust parameters for short input sequences; Expect threshold: 10; Word size: 28; Max matches in a query range: 0; Match/Mismatch Scores: 1, -2; Gap costs: Linear; Filter: Low complexity regions; Mask: Mask for lookup table only. A sequence having an identity score of XX % (for example, 80%) with regard to a reference sequence using the NCBI BLAST version 2.2.31 algorithm with default parameters is considered to be at least XX % identical or, equivalently, have XX % sequence identity to the reference sequence.

As used herein, the terms “lactate,” “lactic acid,” and the like, are used interchangeably unless specifically noted otherwise, and can refer to lactic acid, the conjugate base of lactic acid (lactate), or salts thereof. It is contemplated herein that lactic acid is primarily in the form of the conjugate base when utilized by the organisms described herein, but that the lactic acid or salt forms can also be present and/or utilized by the organisms, as would be understood by a person skilled in the art. Further, “lactate” or “lactic acid” can refer to D-Lactate, L-Lactate, or a mixture thereof. Unless either D- or L-lactate is specifically recited herein, the term “lactate” refers to a mixture of D- and L-enantiomers.

As used herein, the term “hexose” refers to a 6-carbon sugar or a mixture of 6-carbon sugars. In some embodiments, the hexose is gluconse or is a mixture of sugars that includes gluconse. Throughout this disclosure, any description that refers specifically to gluconse alone is also applicable to any hexose or mixture of hexoses.

As used herein, the terms “monocarboxylic transporter,” “monocarboxylate transporter,” “monocarboxylate/proton symporter,” “monocarboxylic acid permease,” “monocarboxylate permease,” “carboxylic acid permease,” and the like are used interchangeably, and refer to transporters that carry monocarboxylate molecules, such as lactate and pyruvate, across cell membranes.

Throughout this disclosure, various aspects of the invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 7 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 6, from 2 to 5, from 3 to 5, etc., as well as individual numbers within that range, for example, 1, 2, 3, 3.6, 4, 5, 5.8, 6, 7, and any whole and partial increments in between. This applies regardless of the breadth of the range.

Description

Described herein are genetically modified organisms, methods for producing such organisms, and fermentation processes using such organisms. The organisms can consume lactate during a fermentation process while producing a bioproduct. In one aspect, the organisms can consume lactate in the presence of gluconse while producing a bioproduct. Therefore, the present invention relates to organisms and processes that can use lactate as a carbon source for fermentation instead of, or in addition to, carbohydrates. Accordingly, the organisms and processes are useful for fermenting substrates containing lactate, such as corn or other vegetable processing streams.

Many fermentation substrate feed streams, including corn process streams, contain some amount of lactate. For example, the growth of lactate-producing *Lactobacilli* is often promoted in the steeping step of wet corn milling processes to assist in the breakdown of the corn into its various components. However, most or all of the lactate remains unconsumed in the fermentation broth in currently available fermentation processes. Therefore, the processes and organisms of the present invention can provide a benefit over currently available processes or organisms by consuming a higher percentage of the available carbon sources in the feed stream, resulting in improved fermentation yields, production rates, and/or efficiency.

Genetically Modified Yeasts and Methods for Producing Such Yeasts

In one aspect, the present invention relates to genetically modified yeasts and methods for producing such yeasts. In some embodiments, the genetically modified yeast is of the genus *Saccharomyces*. In some embodiments, the yeast is a genetically modified *Saccharomyces cerevisiae* yeast. In one aspect, the yeast is genetically modified to include one or more traits that enable the yeast to consume lactate or improve the lactate consumption capability of the yeast while producing a fermentation product. In some embodiments, the yeast can consume lactate at a faster rate than a yeast without the modification(s) described herein, espe-

cially when hexose or hexose oligomers such as gluconse are present in the fermentation broth. In some embodiments, the yeast can consume D-lactate, L-lactate, or both D-lactate and L-lactate at a faster rate than a yeast without the modification(s) described herein. In some embodiments, the yeast can produce a bioproduct at a higher titer and/or produce a bioproduct at a faster rate than a yeast without the modification(s) described herein. In some embodiments, the bioproduct is ethanol.

The genetic modifications can include transforming a yeast to contain one or more heterologous genes that relate to lactate consumption or utilization. Non-limiting examples of genetic modifications include: transforming the yeast to overexpress one or more native-type gene(s); transforming the yeast to include multiple copies of one or more native-type gene(s); transforming the yeast with one or more exogenous gene(s); and/or transforming the yeast to include multiple copies of one or more exogenous gene(s).

In one aspect, the genetic modifications can relate to lactate transport, for example transforming a yeast to express a heterologous monocarboxylate/proton symporter gene. In some embodiments, the monocarboxylate/proton symporter gene is from a yeast of the genus *Kluyveromyces*. In some embodiments, the monocarboxylate/proton symporter gene is a *K. Lactis* (SEQ ID NO: 27), a *K. marxianus* (SEQ ID NO: 73), or a *K. dobzhanskii* (SEQ ID NO: 71) JEN1 symporter gene. In some embodiments, the monocarboxylate/proton symporter gene is from a yeast of the genus *Yarrowia*, for example *Yarrowia lipolytica* (SEQ ID NO: 79). In some embodiments, the monocarboxylate/proton symporter gene is from a genus of yeast other than genus *Kluyveromyces* or *Yarrowia*, for example, but not limited to, *Saccharomyces* or *Issatchenkia*. In some embodiments, the monocarboxylate/proton symporter gene is from *S. cerevisiae* or *I. orientalis* (SEQ ID NO: 23, SEQ ID NO: 25). In some embodiments, the genetically modified yeast encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to at least one of the following amino acid sequences: SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, or SEQ ID NO: 78. In some embodiments, the yeast encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 70%, 74% or 75% to either the *K. Lactis* (SEQ ID NO: 28) or *K. dobzhanskii* (SEQ ID NO: 76) JEN1 polypeptides, or both.

In some embodiments, the genetic modifications can relate to lactate consumption or utilization. In some embodiments, the yeast is transformed to express one or more heterologous genes encoding a lactate dehydrogenase (cytochrome) (EC 1.1.2.3 or 1.1.2.4). In some embodiments, the yeast is transformed to express a heterologous cytochrome b2 (CYB2) gene. In some embodiments, the CYB2 gene is a *S. cerevisiae* (SEQ ID NO: 13) or *I. orientalis* CYB2 (SEQ ID NO: 16) gene. In some embodiments, the CYB2 gene is from *S. kluyveri* (SEQ ID NO: 53), *S. bayanus* (SEQ ID NO: 55), *Z. rouxii* (SEQ ID NO: 57), *K. lactis* (SEQ ID NO: 59), or *K. dobzhanskii* (SEQ ID NO: 61). In some embodiments, the genetically modified yeast having a heterologous CYB2 gene encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to at least one of the following amino acid sequences: SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62.

In some embodiments, the yeast is transformed to express a heterologous D-lactate dehydrogenase (DLD) gene. In

some embodiments, the DLD gene is a *S. cerevisiae* (SEQ ID NO: 39) or *I. orientalis* DLD (SEQ ID NO: 41) gene. In some embodiments, the DLD gene is from *S. kluyveri* (SEQ ID NO: 63), *S. bayanus* (SEQ ID NO: 65), *A. fumigatus* (SEQ ID NO: 67), *K. lactis* (SEQ ID NO: 69), *K. dobzhanskii* (SEQ ID NO: 71), or *K. marxianus* (SEQ ID NO: 73). In some embodiments, the genetically modified yeast having a heterologous DLD gene encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to at least one of the following amino acid sequences: SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, or SEQ ID NO: 74. In some embodiments, the yeast is transformed to express both a heterologous CYB2 gene and a DLD gene.

In one aspect, the genetically modified yeast can be transformed to include any combination of heterologous monocarboxylate/proton symporter genes and lactate dehydrogenase genes. In some embodiments, the yeast contains a heterologous monocarboxylate/proton symporter gene, a heterologous CYB2 gene, and a heterologous DLD gene. In some embodiments, the yeast contains a heterologous monocarboxylate/proton symporter gene and a heterologous CYB2 gene. In some embodiments, the yeast contains a heterologous monocarboxylate/proton symporter gene and a heterologous DLD gene. In some embodiments, the yeast contains more than one copy of any of a heterologous monocarboxylate/proton symporter gene, a heterologous CYB2 gene, and/or a heterologous DLD gene, i.e., the yeast can be transformed with two or more copies of a heterologous monocarboxylate/proton symporter gene; two or more copies of a heterologous CYB2 gene; and/or two or more copies of a heterologous DLD gene. Further, the yeast can be modified to contain heterologous genes from any combination of species, for example, a *S. cerevisiae* yeast can be modified to contain one or more copies of a *K. lactis* JEN1, one or more copies of a heterologous *S. cerevisiae* CYB2, and one or more copies of a heterologous *S. cerevisiae* DLD. Accordingly, the yeast can encode for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to one, two, three, or more of a monocarboxylate/proton symporter-related, CYB2-related, and/or DLD-related amino acid sequence, for example at least one of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, or SEQ ID NO: 74.

While not wishing to be bound by theory, it is contemplated herein that the lactate consumed by the organism is converted primarily to pyruvate, and at least a portion of the pyruvate is converted to the desired fermentation product. Even lactate that is consumed by the organism for respiration can provide a benefit because the organism is using lactate instead of other potentially preferred carbon sources such as gluconse.

Strains of *Saccharomyces cerevisiae* that can consume D-lactate and produce ethanol have been described (Wakamatsu et al., 2013, Biosci. Biotechnol. Biochem. 77(5) 1114-1116). However, the strains described in Wakamatsu produced ethanol from a fermentation medium containing only lactate, and not from a medium that also contains gluconse. Further, Andrade described that JEN1 expression is inhibited by the presence of gluconse (Andrade et al.,

2001, Fungal Genetics and Biology 32, 105-111). Therefore, currently available yeast strains cannot consume significant amounts of lactate and gluconse simultaneously while producing a fermentation product at a commercially useful rate. Further, it is known that many yeasts produce some lactic acid rather than consuming lactic acid during fermentation. However, the present invention relates to genetically modified yeasts that can surprisingly use both lactate and gluconse as a carbon source simultaneously to produce a fermentation product at rates suitable for commercial applications. In one aspect, the genetically modified yeast can consume L-lactate at significantly higher rates than currently available yeast, whether or not gluconse or another hexose is present.

In one aspect, the yeast can be genetically modified to consume D-lactate, L-lactate, or a mixture of D- and L-lactate. Vegetable processing streams that contain lactate typically contain a mixture of D-lactate and L-lactate enantiomers, for example a racemic mixture or a mixture containing some ratio of D- and L-lactate other than 50:50. In one aspect, the yeast can be genetically modified to contain a heterologous lactate racemase-expressing gene. A yeast that contains a racemase expressing gene can be used to improve the lactate-consuming characteristics of the yeast. For example, a genetically modified yeast according to the present invention that can consume D-lactate more quickly than L-lactate can be modified to express a lactate racemase. As the D-lactate is consumed more quickly by the yeast, the racemase can be used to convert L-lactate to D-lactate to improve the overall rate of lactate consumption.

As described above, the yeast can include heterologous JEN1, DLD, and/or CYB2 gene(s), resulting in encoding polypeptides having a minimum sequence identity to certain amino acid sequences listed herein. In some embodiments, signature patterns at positions corresponding to specific residue ranges have been identified. In some embodiments, the yeast of the present invention is transformed with a construct having these patterns conserved. Accordingly, in some embodiments, the yeast can encode for polypeptides having a signature pattern at certain amino acid residue positions.

In one aspect, the genetically modified yeast encodes for a CYB2 protein having the following amino acid residues conserved at the specified positions: Lys349, Tyr143, Tyr254 and His373. Xia describes the involvement of these residues in the catalytic oxidation of lactate to pyruvate (Xia and Mathews, 1990, J. of Molecular Biology. 212 pp. 837-863, which is hereby incorporated by reference in its entirety). In some embodiments, the yeast of the present invention encodes a polypeptide having a sequence identity of at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% to SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, that includes one or more of the following amino acid residues at the indicated positions: Lys349, Tyr143, Tyr254 and His373. The amino acids Lys349, Tyr143, Tyr254 and His373 have a role in the mechanism of lactate oxidation via interaction with pyruvate. In some embodiments, such a yeast includes a heterologous CYB2 gene from any of the following species: *S. bayanus*, *S. kluyveri*, *Zygosaccharomyces rouxii*, *K. lactis*, *K. dobzhanskii*, *K. marxianus*, *I. orientalis*, *Aspergillus fumigatus*, or *A. niger*. In some embodiments, such a yeast also includes at least one heterologous monocarboxylate/proton symporter gene and encodes for a polypeptide having a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% to SEQ ID NO: 24,

SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, or SEQ ID NO: 78. In some embodiments, such a yeast also includes at least one heterologous DLD gene and encodes for a polypeptide having a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% to SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, or SEQ ID NO: 74.

In one aspect, the genetically modified yeast can consume D-lactate, L-lactate, or a mixture thereof at a faster rate than currently available yeasts. In some embodiments, the genetically modified yeast can consume D-lactate and/or L-lactate, in conditions where currently available yeasts cannot consume D-lactate and/or L-lactate. In one aspect, it is useful to measure the lactate consumption rate according to the Evaluation Protocol for Lactate Consumption described later herein. In some embodiments, the yeast has a L-lactate consumption rate of at least 0.030 gL⁻¹h⁻¹ when the concentration of hexose is 150 g/L or greater. In some embodiments, the yeast has a L-lactate consumption rate of at least 0.035, 0.040, 0.045, or 0.050 gL⁻¹h⁻¹ when the concentration of hexose is 150 g/L or greater. In some embodiments, the yeast has a L-lactate consumption rate of at least 0.015, 0.020, 0.025, 0.030, 0.035, or 0.040 gL⁻¹h⁻¹ when the concentration of hexose is 5 g/L or greater. In some embodiments, the yeast has a D-lactate consumption rate of at least 0.005, 0.006, 0.007, 0.008, 0.009, 0.010, 0.015, 0.020, 0.025, or 0.030 gL⁻¹h⁻¹ when the concentration of hexose is 150 g/L or greater. In some embodiments, the yeast has a D-lactate consumption rate of at least 0.015, 0.020, 0.025, 0.030, 0.035, or 0.040 gL⁻¹h⁻¹ when the concentration of hexose is 5 g/L or greater.

In some embodiments, the yeast can include other genetic modifications. In one aspect, the yeast can be transformed to express enzymes useful for improving the production of a bioproduct. In one aspect, the yeast can be transformed to contain genes related to expressing enzymes that are typically added to a fermentation process, for example, enzymes used to generate gluconse or another hexose from hexose oligomers. In one aspect, the yeast can be transformed to reduce or eliminate the conversion of the carbon source(s) to undesired compounds. In some embodiments, the yeast can include one or more genetic modifications useful for improving any other aspects of an ethanol production process. Non-limiting examples of genetic modifications that can be useful for an ethanol-producing yeast include transforming the yeast to express a heterologous gene for producing glucoamylase (EC 3.2.1.3), for example the yeast expresses a glucoamylase from *Saccharomycopsis fibuligera* or *Rhizopus oryzae*; transforming the yeast to express a heterologous gene for consuming isomaltose; and/or transforming the yeast to reduce the formation of glycerol in the yeast during fermentation. In some embodiments, the yeast can be transformed to secrete a heterologous lactate oxidase. Further, the yeast can be transformed to secrete heterologous catalase in addition to the lactate oxidase to consume hydrogen peroxide.

In some embodiments, the genetic modifications to the yeast associated with increasing the consumption of lactate (e.g., a yeast modified to include a heterologous CYB2 gene, DLD gene, and/or JEN1 gene) can also relate to the reduction of glycerol produced by the yeast during fermentation. The reduction of glycerol produced during fermentation can increase the amount of carbon transformed from the carbon source(s) (e.g., gluconse and/or lactate) into the fermentation product. Accordingly, the yeast can produce a higher yield of a fermentation product, such as ethanol, and gen-

erate a lower amount of glycerol than a yeast that does not include the genetic modifications associated with increased lactate consumption.

In some embodiments, the yeast can be further engineered by a method other than genetic modification. In some embodiments, after the yeast is genetically modified as described above, the yeast is evolved. In some such embodiments, the evolution can include propagating the yeast for multiple generations to allow for the yeast to naturally select for certain characteristics.

In some embodiments, the yeast can be modified by mutagenesis. In some such embodiments, the yeast can be selected for certain characteristics after mutagenesis. In some embodiments, the mutagenesis can include subjecting the yeast to ultraviolet radiation and/or chemical treatment. In some embodiments, the selected characteristics of the yeast can include heat tolerance, ethanol tolerance, inhibitor tolerance, improved growth rate, and/or by-product reduction.

Accordingly, in one aspect, the present invention also relates to a method for evolving a yeast that can consume lactate while producing a fermentation product. In some embodiments, the method includes the steps of: transforming a yeast to express one or more heterologous genes associated with increased lactate consumption, then evolving the yeast and selecting the yeast based on a desired characteristic. In some embodiments, the method includes the steps of: transforming a yeast to express one or more heterologous genes associated with increased lactate consumption, exposing the yeast to an environment capable of causing mutagenesis, and selecting the yeast for one or more desired characteristics. In one aspect, the method for evolving the yeast can use any yeast described herein, i.e., a genetically modified yeast transformed to increase lactate consumption during fermentation.

In one aspect, the non-natural yeast of the present invention has the ability to grow, and/or can produce a fermentation product at temperatures that are greater than those in which yeast, for example *Saccharomyces cerevisiae*, typically are used in fermentation processes. For example, *S. cerevisiae* typically have optimal growth at a temperature of about 30° C. In some embodiments, the yeast has a greater tolerance to elevated temperatures, such as 32° C. or greater, such as in the range of greater than 32° C. to about 40° C. Exemplary ranges for elevated temperature are T_1 to T_2 , wherein T_1 is selected from 32.2° C., 32.4° C., 32.6° C., 32.8° C., 33° C., 33.2° C., 33.3° C., 33.4° C., 33.6° C., 33.8° C., 34° C., 34.2° C., 34.4° C., 34.6° C., 34.8° C., 35° C., and 36° C.; and T_2 is selected from 36° C., 37° C., 38° C., 39° C., and 40° C. For the purposes of this disclosure, a yeast is considered "thermotolerant" if the yeast can continue to grow, reproduce, and/or produce a fermentation product during or after being exposed to a fermentation medium having an elevated temperature.

During a fermentation process the fermentation medium can reach an elevated temperature of 32° C. or greater during one or more time(s) during the fermentation process. The temperature can be elevated during part of the fermentation period, or during the entire fermentation period. The temperature can be elevated for 5 minutes or greater, 10 minutes or greater, 30 minutes or greater, 1 hour or greater, 2 hours or greater, 5 hours or greater, or 10 hours or greater. The time of elevated temperature can also be expressed as a total of the overall fermentation period, such as about 0.1% to 100%, about 0.1% to about 75%, about 0.1% to about 50%, about 0.1% to about 25%, about 0.1% to about 10%, about

0.1% to about 5%, about 0.1% to about 2.5%, about 0.1% to about 1%, or about 0.1% to about 0.5% of the fermentation period.

The engineered yeast can also provide a commercially relevant titer of ethanol during or after the period of elevated temperature. For example, during or after the period of elevated temperature, for example, any of the ranges corresponding to T_1 to T_2 , the ethanol titer can be in the range of about 110 g/L to about 170 g/L, in the range of about 125 g/L to about 170 g/L, or in the range of about 140 g/L to about 170 g/L. Accordingly, the engineered yeast described herein can produce ethanol at a commercially useful titer during or after a period of high temperature that would typically cause issues in other currently available yeast strains used in ethanol-producing fermentation processes. Such issues include but are not limited to: death to a significant percentage of yeast cells; deleterious effects on the ability of the yeast to reproduce; and/or reduction or elimination of the ability of the yeast to produce a fermentation product.

An engineered *S. cerevisiae* described herein can be put under temperature selection pressure to select for strains that demonstrate increased tolerance to growth at higher temperatures. The engineered yeast can be subjected to random mutagenesis (e.g., UV, chemical) prior to application of the higher temperature selection to generate mutation(s) that can confer improved tolerance to growth at these higher temperatures. For example, an engineered yeast of the disclosure can have a specific growth rate at a temperature in the range of 32° C. or greater that is 10%, 20%, 30%, 40%, or 50% greater than the growth rate of a reference yeast. In some embodiments, the reference yeast is a yeast expressing a *Saccharomycopsis fibuligera* glucoamylase that is capable of producing ethanol at a titer of at least 90 g/L. In one aspect, this disclosure also relates to a method for producing a yeast product suitable for use in a fermentation process. In some embodiments, such a method includes the steps of growing a yeast, for example any of the engineered yeasts described herein, and isolating the yeast from the medium used to grow the yeast. The method can further include the step of drying the yeast to form a yeast product. Drying the yeast can improve the shelf-life of the yeast. In some embodiments, the dried yeast has a moisture content of less than 5%, less than 4%, less than 3%, less than 2%, or less than 1%.

45 Fermentation Processes

In one aspect, the present invention relates to fermentation processes. In one aspect, the fermentation processes can be any process using an embodiment of the genetically modified yeasts described herein to produce a fermentation product. In some embodiments, the fermentation product is ethanol. In some embodiments, the fermentation product can be an alcohol other than ethanol, for example, but not limited to, n-propanol, iso-propanol, n-butanol, iso-butanol, butadiene, or isoprene.

An exemplary fermentation process can include the steps of providing a fermentation medium that contains a carbon source, adding a yeast to the fermentation medium, fermenting the medium with the yeast to produce a bioproduct, and harvesting the bioproduct. In one aspect, the carbon source in the medium can include starches, sugars, organic acids, or a mixture thereof. In some embodiments, the carbon source in the medium is a mixture of gluconse oligomers, gluconse, and/or lactate.

In one aspect, a lactate-containing vegetable process stream is used as the fermentation medium or is added to the fermentation medium at some point in the fermentation process. In one aspect, the processes of the present invention

can be used to ferment a fermentation medium containing a significant amount of lactate in addition to gluconse and/or gluconse oligomers. Non-limiting examples of fermentation media containing a significant amount of lactate include media having at least 0.1, at least 0.5, at least 1, at least 2, at least 3, at least 4, or at least 5 g/L lactate. In some embodiments, the fermentation media can have a lactate content in the range of about 1-20, 2-20, 3-20, 4-20, 5-20, 1-15, 3-15, or 5-15 g/L at the start of fermentation. In some embodiments, the fermentation media can have a lactate content in the range of about 1-50, 15-30, or 20-30 g/L at the start of fermentation. For example, in some embodiments, light steep water (LSW) can contain about 25 g/L lactate.

In some embodiments, the processes described herein can be used to consume most or all of the lactate in the fermentation medium. In some embodiments, the processes can be used to consume only a portion of the lactate in the fermentation medium. The consumption of lactate during the process can depend on a number of factors, including, but not limited to: the characteristics of the yeast used; the rate of oxygen added to the process; the amount of oxygen present at the start of fermentation; the gluconse concentration in the fermentation medium, pH, and temperature.

In one aspect, as would be understood by a person skilled in the art, the composition of the medium can vary during fermentation. For example, gluconse or another hexose can be generated from oligomers during fermentation via enzymatic activity, then consumed. Accordingly, in some embodiments, the gluconse content can be very low or even undetectable at some points of the fermentation if gluconse is consumed by the yeast faster than it is generated from the gluconse oligomers. In some embodiments, for example fed-batch fermentation, the medium can be continuously or semi-continuously supplemented with a feed stream, such as a vegetable process feed stream. In some embodiments, the lactate content can be very low or even undetectable at some points of the fermentation due to consumption by the yeast, and then more lactate can be added to the medium via a vegetable process feed stream.

Accordingly, in one aspect, the concentrations of various components of the fermentation medium for the processes described herein can be an average concentration. Average concentrations of components can be calculated via known methods in the art, for example by taking the average of the concentration of a component in the fermentation medium at the start of fermentation and the concentration of the same component in the fermentation medium of the end of fermentation. Such a calculation of average can also account for the concentration of the component in any input and/or output streams during the fermentation process. Further, for a continuous fermentation process, the average concentration of a component can refer to the average concentration in any single vessel, or it can refer to the average concentration over the entire process, i.e., accounting for all feed streams and all output streams of the process.

In some embodiments, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of the lactate present in the fermentation medium and/or added to the fermentation medium is consumed by the end of fermentation. In some embodiments, the rate of lactate consumption during fermentation is in the range of 0.01 to 1.5 g L⁻¹ h⁻¹, 0.05 to 1 g L⁻¹ h⁻¹, or 0.1 to 1 g L⁻¹ h⁻¹. In some embodiments, the rate of lactate consumption during fermentation is at least 0.01 g L⁻¹ h⁻¹, 0.05 g L⁻¹ h⁻¹, 0.1 g L⁻¹ h⁻¹, 0.5 g L⁻¹ h⁻¹, or 1 g L⁻¹ h⁻¹. In some embodiments, the rates of lactate consumption listed above can refer to the average lactate

consumption during fermentation. In some embodiments, the rates of lactate consumption listed above can refer to the lactate consumption during the first portion of the fermentation process, for example, over the first 10 hours, 8 hours, 6 hours, 4 hours, 2 hours, 1 hours, or 30 min of the fermentation process. In some embodiments, the rates of lactate consumption listed above refer to the rate of lactate consumption when gluconse is present. In some embodiments, the gluconse concentration is at least 0.1 g/L, 1 g/L, 10 g/L, 20 g/L, or 30 g/L when the rate of lactate consumption during fermentation is at least 0.01 g L⁻¹ h⁻¹, 0.05 g L⁻¹ h⁻¹, 0.1 g L⁻¹ h⁻¹, 0.5 g L⁻¹ h⁻¹, or 1 g L⁻¹ h⁻¹.

In some embodiments, the amount of total lactate in the fermentation medium at the end of fermentation is in the range of 0 to 3 g/L, 0 to 1 g/L, 0 to 0.5 g/L, 0 to 0.1 g/L, 0.001 to 3 g/L, 0.001 to 1 g/L or 0.001 to 0.1 g/L. In some embodiments, the amount of total lactate in the fermentation medium at the end of fermentation is less than 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, or 0.1 g/L. In some embodiments, the amount of L-lactate in the fermentation medium at the end of fermentation is in the range of 0 to 3 g/L, 0 to 1 g/L, 0 to 0.5 g/L, 0 to 0.1 g/L, 0.001 to 1 g/L or 0.001 to 0.1 g/L. In some embodiments, the amount of L-lactate in the fermentation medium at the end of fermentation is less than 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, or 0.1 g/L. In some embodiments, the amount of D-lactate in the fermentation medium at the end of fermentation is in the range of 0 to 3 g/L, 0 to 1 g/L, 0 to 0.5 g/L, 0 to 0.1 g/L, 0.001 to 1 g/L or 0.001 to 0.1 g/L. In some embodiments, the amount of D-lactate in the fermentation medium at the end of fermentation is less than 3 g/L, 2 g/L, 1 g/L, 0.5 g/L, or 0.1 g/L.

In one aspect, the process of the present invention can be used to produce ethanol at commercially significant rates and/or titers. In some embodiments, the rate of ethanol produced can be 1 to 6 g L⁻¹ h⁻¹, 1 to 5.5 g L⁻¹ h⁻¹, or 1 to 5 g L⁻¹ h⁻¹. In some embodiments, the ethanol titer at the end of fermentation is at least 90 g/L, 100 g/L, 110 g/L, 120 g/L, 125 g/L, 130 g/L, 131 g/L, 132 g/L, 133 g/L, 134 g/L, or at least 135 g/L. In some embodiments, the ethanol titer at the end of fermentation is in the range of 120 g/L to 155 g/L, 130 to 155 g/L, 135 to 155 g/L, 130 to 150 g/L, or 130 to 140 g/L.

In one aspect, the process can use a yeast other than the genetically-modified yeasts described herein, for example the commercially available ETHANOL RED™ yeast (available from Lesaffre/Fermentis), and the process can be run using a high oxygen uptake rate and/or a lower gluconse concentration to promote lactate consumption.

In one aspect, the process can be used to consume L-lactate, D-lactate, or a mixture of L- and D-lactate. In one aspect, the process can include using a lactate racemase to improve the lactate-consuming characteristics of the yeast. In some embodiments, the yeast used in the process can be a genetically-modified yeast that secretes a lactate racemase. In some embodiments, the exogenous lactate racemase can be added to process instead of, or in addition to, lactate racemase secreted by the yeast. Other exogenous enzymes can be added to the process. For example, in some embodiments an exogenous lactate oxidase can be added to the process. In one such embodiment, exogenous catalase can be added to the process to decompose hydrogen peroxide formed by the activity of the exogenous lactate oxidase.

Batch Fermentation Processes

In one aspect, the process of the present invention can be a batch fermentation process. In some embodiments, the batch process of the present invention is a dry-grind or dry-milling ethanol production process. Batch fermentation

processes, including dry-grind ethanol processes are well-known in the art. As would be understood by a person skilled in the art, lactate can be formed during the dry milling of corn or other vegetable matter, for example, as a result of bacterial contamination. In one aspect, the genetically modified yeast of the present invention can be used in a batch fermentation process to produce a fermentation product using lactate as a carbon source.

An exemplary batch fermentation process includes the steps of providing a fermentation medium that contains lactate and/or other carbon sources such as carbohydrates and fermenting the medium using a genetically modified yeast of a type described herein. In some embodiments, the yeast contains a heterologous monocarboxylate/proton symporter (e.g., JEN1) gene. In some embodiments, the medium contains gluconse or gluconse oligomers at concentration of at least 0.5, 1, 2, or 3 g/L at the start of fermentation. In some embodiments, the lactate concentration is at least 0.5, 1, 2, 3, 4, 5, or 6 g/L at the start of fermentation. In some embodiments, the lactate concentration is in the range of 1 to 20 g/L, 1 to 15 g/L, 1 to 10 g/L, 1 to 8 g/L, or 1 to 5 g/L at the start of fermentation. In some embodiments, at least 20, 30, 40, 50, 60, 70, 80, 90, 95, or 99% of the total lactate is consumed at the end of fermentation. In some embodiments, at least 50, 60, 70, 80, 90, 95, or 99% of the D-lactate is consumed at the end of fermentation. In some embodiments, at least 50, 60, 70, 80, 90, 95, or 99% of the L-lactate is consumed at the end of fermentation.

In one aspect, the batch fermentation process is run with a minimum volumetric oxygen uptake rate (OUR) for at least a part of the process. In some embodiments, the OUR is at least 0.5, 1.0, or 1.5 mmol O₂/(L·h) during at least part of the process. In some embodiments, the OUR is in the range of 0.5 to 5.0 mmol O₂/(L·h) during at least part of the process. In some embodiments, the OUR is in the range of 3.5 to 4.5 mmol O₂/(L·h) during at least part of the process. In some embodiments, the OUR is at least 0.5, 1.0, or 1.5 mmol O₂/(L·h) for the initial part of the process. In some embodiments, the OUR is at least about 0.5 or 1.0, or 1.5 mmol O₂/(L·h) for the first 6, 8, or 10 hours of the process. In some embodiments, the OUR is reduced or about zero during part of the process. In some embodiments, the OUR is less than 0.5 or 1.0 mmol O₂/(L·h) after about the first 6, 8, 10, 12, 15, or 20 hours of the process.

Continuous Fermentation Processes

In one aspect, the process of the present invention can be a continuous or a semi-continuous fermentation process. In some embodiments, the continuous process of the present invention is a wet corn milling ethanol production process. Continuous fermentation processes, including wet milling ethanol processes are well-known in the art. In some embodiments, a fermentation process having a continuous mode of operation includes multiple fermenters that operate in series in which a starch hydrolysate is supplied in the first fermenter, which is fed to second fermenter and so on until the starch hydrolysate is converted to ethanol. In some embodiments, continuous operation can be operated using between 1 to 10 or 2 to 7 fermenters. In some embodiments, a continuous fermentation process can be performed in a single vessel, in which feedstock can be added and product-containing broth can be removed on a continuous or semi-continuous schedule.

An exemplary continuous fermentation process for manufacturing ethanol comprises the following steps: providing an initial fermentation medium that contains gluconse or gluconse oligomers, fermenting the fermentation medium with a genetically modified yeast, adding one or more feed

streams comprising lactate to the fermentation medium, and removing at least one output stream comprising ethanol from the fermentation medium. In some embodiments, the initial fermentation medium can contain lactate and/or other carbon sources in addition to gluconse or gluconse oligomers. In one aspect, the genetically modified yeast is a yeast according to any of the embodiments of a genetically modified yeast described herein. In some embodiments, the yeast contains a heterologous monocarboxylate/proton symporter (e.g., JEN1) gene.

In some embodiments, the initial fermentation medium is added to a pre-fermenter or growth fermenter vessel, where the genetically modified yeast is added and grown until a desired biomass is achieved. In some embodiments, the conditions of the process in the pre-fermenter are set to favor cell growth over fermentation product formation. In some embodiments, the contents of the pre-fermenter vessel can then be transferred to a second fermenter vessel. In the second fermenter vessel, the conditions of the process are set to favor the formation of fermentation product over cell growth. In some embodiments, additional fermentation medium is added to the second fermenter vessel, either in a single portion or in a continuous or semi-continuous manner. In some embodiments, the additional fermentation medium added to the second fermenter vessel contains lactate and/or other carbon sources. The second fermenter referred to above can also be referred to as a "propagator." In some embodiments, the contents of the second fermenter vessel can be transferred to a third fermenter vessel. The process conditions of the third fermenter vessel can be the same or different as the second fermenter vessel. In some embodiments, the contents of third fermenter vessel can be transferred to one or more additional fermenter vessels, as would be understood by a person skilled in the art of continuous fermentation processes. In some embodiments, the bioproduct, e.g., ethanol, is isolated from the contents of the final fermenter vessel.

In some embodiments, the average gluconse concentration of the fermentation medium in the pre-fermenter vessel is in the range of 10 to 20 g/L. In some embodiments, the average gluconse concentration of the fermentation medium in the second fermenter vessel is in the range of 30 to 40 g/L. In some embodiments, the average gluconse concentration of the fermentation medium in the third fermenter vessel, or any additional fermenter vessel, is in the range of 30 to 40 g/L. In some embodiments, the average gluconse concentration of the fermentation medium in the final fermenter vessel is in the range of 0 to 5 g/L. In some embodiments, the average gluconse concentration of the fermentation medium in the pre-fermenter vessel, propagator vessel, or in any of the fermentation vessels is in the range of 0-5, 2-5, 1-10, 5-10, 5-15, 5-20, 10-20, 15-25, 20-30, 25-35, 30-40, or 35-45 g/L. In some embodiments, the average gluconse concentration of the fermentation in the pre-fermenter vessel, propagator vessel, or in any of the fermentation vessels is maintained in a range that is greater than or equal to the gluconse concentration associated with gluconse repression in a yeast. In some embodiments, the gluconse concentration associated with gluconse repression in a yeast is in the range of 2 to 5 g/L. Accordingly, in such embodiments, the average gluconse concentration of the fermentation medium in the pre-fermenter vessel or in any of the fermentation vessels can be maintained at a level greater than or equal to 2, 3, 4, or 5 g/L.

Other fermentation conditions can be adjusted and/or maintained in the continuous fermentation process, including, but not limited to: temperature, pH, volumetric or

specific oxygen uptake rate (OUR), or the concentration of any carbon source or any fermentation medium nutrient. In some embodiments, the temperature in the pre-fermenter vessel, propagator vessel, or in any other fermentation vessel can be in the range of 20-45, 20-40, 20-30, 25-35, or 30-40° C. In some embodiments, the pH in the pre-fermenter vessel, propagator vessel, or in any other fermentation vessel can be in the range of 2 to 7, 3 to 6, 4.5 to 5.5, or 3.5 to 4.5. As would be understood by a person skilled in the art, the pH at the start of fermentation can be lower than the pH at the end of fermentation as a result of lactate being consumed by the yeast. Accordingly, the pH at the end of fermentation can be in a different range of those listed above than the pH at the start of fermentation. In some embodiments, the volumetric OUR in the pre-fermenter vessel, propagator vessel, or in any other fermentation vessel is at least 0.5 mmol O₂/(L·h) or at least 1 mmol O₂/(L·h). In some embodiments, the volumetric OUR in the pre-fermenter vessel, propagator vessel, or in any other fermentation vessel is in the range of 0.1 to 5 mmol O₂/(L·h), 3 to 5 mmol O₂/(L·h), 0.2 to 1 mmol O₂/(L·h), 0.4 to 0.6 mmol O₂/(L·h), 4 to 200 mmol O₂/(L·h), 8 to 10 mmol O₂/(L·h), or 4 to 30 mmol O₂/(L·h). In some embodiments, the volumetric OUR in the pre-fermenter vessel, propagator vessel, or in any other fermentation vessel is at least 4 mmol O₂/(L·h), at least 5 mmol O₂/(L·h), at least 6 mmol O₂/(L·h), at least 7 mmol O₂/(L·h), or at least 8 mmol O₂/(L·h).

In some embodiments, the cell density in the pre-fermenter vessel is in the range of 3 to 10 g/L or 5 to 10 g/L. In some embodiments, the cell density in the propagator vessel is in the range of 10 to 50 g/L.

In one aspect, as described herein, the yeast consumes lactate during the fermentation process. In some embodiments, the total lactate content in the sum of all output streams is less than 90% of the lactate added to the fermentation process, i.e., the lactate content in sum of all input or feed streams. In some embodiments, the total lactate content in the output of the fermentation process is less than 99%, 95%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, or 10% of the input to the fermentation process. In some embodiments, the total D-lactate content in the output of the fermentation process is less than 99%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, or 10% of the input to the fermentation process. In some embodiments, the total L-lactate content in the output of the fermentation process is less than 99%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, or 10% of the input to the fermentation process.

The continuous fermentation processes described herein can produce ethanol or another bioproduct at commercially significant rates. In some embodiments, the processes can produce the bioproduct at a rate of at least 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, or 3.2 g L⁻¹ h⁻¹.

Advantages in Producing Certain Bioproducts

In one aspect, the lactate-consuming characteristics of the yeast can provide advantages in producing certain bioproducts, or in certain fermentation processes. The genetically modified yeasts and fermentation processes described herein can be used to make ethanol or other bioproducts of interest. Other bioproducts, include alcohols other than ethanol, lactic acid, 3-hydroxypropionic acid (3-HP), and succinic acid.

In some embodiments, the yeasts described herein can be useful for making enantiomerically-pure lactic acid. For example, by consuming one enantiomer with relatively high

specificity, the yeast can produce D-lactic acid or L-lactic acid having enantiomeric purities of greater than 95%, 97%, 98%, 99%, 99.5% or more.

In some embodiments, the yeasts can improve the production of organic acids such as succinic acid or 3-HP. For example, it can be problematic to separate lactic acid from an organic acid product when recovering the organic acid product from the fermentation broth in downstream processing. By consuming lactate, the yeasts described herein can improve the efficiency of downstream processing and/or improve the purity of the final organic acid product.

In one aspect, the yeasts described herein can enable the use of vegetable process streams in fermentation processes that cannot typically use such streams. Vegetable process streams, such as corn steep liquor, are not typically used to produce bioproducts other than ethanol due to impurities such as lactate present in these streams. By consuming the lactate, the yeasts of the present invention can enable vegetable process streams to be used in a wide variety of fermentation processes.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Evaluation Protocol for Lactate Consumption

The protocol described below is used to determine the consumption of L-lactate, D-lactate, or a mixture of D- and L-lactate (i.e., "total lactate") by a yeast strain.

A yeast strain is selected and streaked out on a ScD-Ura plate and incubated at 30° C. until single colonies are visible (1-2 days) or grown overnight in 4 ml of YP+100 g/L D-gluconse in a 14 ml culture tube as a liquid culture at 30° C./250 rpm to generate seed biomass. A shake flask is inoculated with seed cell slurry to reach an initial OD₆₀₀ of 0.1-0.3. Immediately prior to inoculating, 60 mL of shake flask medium is added to a 250 mL non-baffled shake flask (Corning 4995-250) fitted with a screw cap containing a gas-permeable seal (Corning 1395-45LTMC). The shake flask medium is prepared according to Verduyn (Verduyn, et al., 1992, Yeast. Vol. 8, 501-517, which is hereby incorporated by reference in its entirety), with the exception of substituting manganese tetrahydrate with manganese dihydrate. The medium is supplemented with D-gluconse to target an initial D-gluconse concentration. For example, the medium is supplemented with 250 grams of D-gluconse per liter of media. To evaluate L-lactate consumption only, sodium L-lactate (e.g., Sigma; catalog number L7022) is used. To evaluate D-lactate consumption only, sodium D-lactate (e.g., Sigma; catalog number 71716) is used. To evaluate a mixture of D- and L-lactate, a mixture of about 50% D-lactate and 50% L-lactate is used. The pH of the media is adjusted to pH 4.5, and sterile filtered prior to use. The inoculated flask is incubated at 30° C. with shaking in an orbital shake at 75 rpm for at least 40 hours. Samples are taken and analyzed for gluconse, ethanol, and lactate concentration in the broth during fermentation using high performance liquid chromatography with a refractive index detector.

A lactate consumption rate is calculated between two time points: the time of inoculation (t₀) and another time point

later in the fermentation (t_1). The time t_1 is a measure of hours (h) after inoculation. Samples are taken at these two time points and the lactate concentration in both samples is measured. The measured lactate concentration in the sample taken at t_0 is designated $[HLA]_0$ and has units of (gL^{-1}). Likewise, the measured lactate concentration in the sample taken at t_1 is designated $[HLA]_1$ and has units of (gL^{-1}). The lactate consumption rate is then calculated as follows resulting in units of ($\text{gL}^{-1}\text{h}^{-1}$):

$$\text{Lactate consumption rate} = ([HLA]_0) / t_1$$

When D-lactate is measured, the calculation will yield a D-lactate consumption rate. Likewise, when L-lactate is measured, the calculation will yield a L-lactate consumption rate and when total lactate is measured, the calculation will yield a total lactate consumption rate.

For example, a shake flask experiment is sampled at inoculation ($t_0=0$ h) and 48 hours later ($t_1=48$ h). The total lactate in both samples is measured with the following results: $[HLA]_0=25.0 \text{ gL}^{-1}$; and $[HLA]_1=15.9 \text{ gL}^{-1}$. Then total lactate consumption rate is calculated as follows:

$$(25.0-15.9)/48=0.190 \text{ gL}^{-1}\text{h}^{-1}$$

The total lactate consumption rate is $0.190 \text{ gL}^{-1}\text{h}^{-1}$.

Example 1

Generation of Amyolytic *Saccharomyces cerevisiae* Strains

Described below are genetically modified *Saccharomyces cerevisiae* yeast strains. The strains described include strains having genetic modifications that improve the lactate-consuming ability of ethanol producing yeasts.

Strain 1-3: *ura3Δ Saccharomyces cerevisiae* Base Strain

Strain 1 (Ethanol Red™) is transformed with SEQ ID NO: 1. SEQ ID NO: 1 contains the following elements: i) an expression cassette for a mutant version of a 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAH7P) synthase gene from *Saccharomyces cerevisiae* (ARO4-OF1); and ii) flanking DNA for targeted chromosomal integration into the URA3 locus. Transformants were selected on synthetic complete media containing 3.5 g/L of p-fluorophenylalanine, and 1 g/L L-tyrosine (ScD-PFP). Resulting transformants were streaked for single colony isolation on ScD-PFP. A single colony was selected. Correct integration of SEQ ID NO: 1 into one allele of locus A was verified by PCR in the single colony. A PCR verified isolate was designated Strain 1-1.

Strain 1-1 was transformed with SEQ ID NO: 2. SEQ ID NO: 2 contains the following elements: i) an expression cassette for an acetamidase (*amdS*) gene from *Aspergillus nidulans*; and ii) flanking DNA for targeted chromosomal integration into the URA3 locus. Transformants were selected on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 80 mg/L uracil and 1 g/L acetamide as the sole nitrogen source. Resulting transformants were streaked for single colony isolation on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 80 mg/L uracil and 1 g/L acetamide as the sole nitrogen source. A single colony was selected. Correct integration of SEQ ID NO: 2 into the second allele of locus A was verified by PCR in the single colony. A PCR verified isolate is designated Strain 1-2.

Strain 1-2 was co-transformed with SEQ ID NO: 3 and SEQ ID NO: 4. SEQ ID NO:3 contains the following elements: i) an open reading frame for a cre recombinase

from P1 bacteriophage, and ii) flanking DNA homologous to SEQ ID NO:4. SEQ ID NO: 4 contains the following elements: i) a 2 μ origin of replication; ii) a URA3 selectable marker from *Saccharomyces cerevisiae*; and iii) flanking DNA containing a PGK promoter and CYC1 terminator from *Saccharomyces cerevisiae*. Transformants were selected on synthetic dropout media lacking uracil (ScD-Ura). Resulting transformants were streaked for single colony isolation on ScD-Ura. A single colony was selected. The isolated colony was screened for growth on ScD-PFP and Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 80 mg/L uracil and 1 g/L acetamide as the sole nitrogen source. Loss of the ARO4-OF1 and *amdS* genes was verified by PCR. The PCR verified isolate was streaked to YNB containing 5-FOA to select for loss of the 2 μ plasmid. The PCR verified isolate was designated Strain 1-3.

Strain 1-4: *Saccharomyces cerevisiae* Expressing two Codon Optimized Variants of the *Saccharomycopsis fibuligera* Glucoamylase at one Allele of CYB2

Strain 1-3 was co-transformed with SEQ ID NO: 5 and SEQ ID NO: 6. SEQ ID NO:5 contains the following elements: i) DNA homologous to the 5' region of the native CYB2 gene; and ii) an expression cassette for a unique codon optimized variant of the *Saccharomycopsis fibuligera* glucoamylase, under control of the TDH3 promoter and CYC1 terminator; and iii) the URA3 promoter as well as a portion of the URA3 gene. SEQ ID NO: 6 contains the following elements: i) a portion of the URA3 gene and terminator; and ii) an expression cassette for a unique codon optimized variant of the *Saccharomycopsis fibuligera* glucoamylase, under control of the PGK promoter and RPL3 terminator; and iii) DNA homologous to the 3' region of the native CYB2 gene. Transformants were selected on ScD-Ura. Resulting transformants were streaked for single colony isolation on ScD-Ura. A single colony was selected. Correct integration of SEQ ID NO: 5 and SEQ ID NO: 6 at one allele of CYB2 was verified by PCR. The PCR verified isolate was designated Strain 1-4.

Strain 1-5: *Saccharomyces cerevisiae* Expressing Four Codon Optimized Variants of the *Saccharomycopsis fibuligera* Glucoamylase at Both Alleles of CYB2

Strain 1-4 was co-transformed with SEQ ID NO: 7 and SEQ ID NO: 8. SEQ ID NO: 7 contains the following elements: i) DNA homologous to the 5' region of the native CYB2 gene; and ii) an expression cassette for a unique codon optimized variant of the *Saccharomycopsis fibuligera* glucoamylase, under control of the TDH3 promoter and CYC1 terminator; and iii) the TEF1 promoter and a portion of the *Aspergillus nidulans* acetamidase gene (*amdS*). SEQ ID NO: 8 contains the following elements: i) a portion of the *Aspergillus nidulans* acetamidase gene (*amdS*) and ADH1 terminator; and ii) an expression cassette for a unique codon optimized variant of the *Saccharomycopsis fibuligera* glucoamylase, under control of the PGK promoter and RPL3 terminator; and iii) DNA homologous to the 3' region of the native CYB2 gene. Transformants were selected on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 80 mg/L uracil and 1 g/L acetamide as the sole nitrogen source. Resulting transformants were streaked for single colony isolation on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 80 mg/L uracil and 1 g/L acetamide as the sole nitrogen source. A single colony was selected. Correct integration of SEQ ID NO: 7 and SEQ ID NO: 8 at the remaining allele of CYB2 was verified by PCR. The PCR verified isolate was designated Strain 1-5.

Strain 1-6: Recycling the URA3 and amdS markers via cre recombinase in Strain 1-5

Strain 1-5 was transformed with SEQ ID NO: 9. SEQ ID NO: 9 contains the following elements: i) an expression cassette for a mutant version of a 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase gene from *Saccharomyces cerevisiae* (ARO4-OPF); 2) an expression cassette for a cre recombinase from P1 bacteriophage; 3) an expression cassette containing the native URA3, and 4) the *Saccharomyces cerevisiae* CEN6 centromere. Transformants were selected on synthetic complete media containing 3.5 g/L of p-fluorophenylalanine, and 1 g/L L-tyrosine (ScD-PFP). Resulting transformants were streaked for single colony isolation on ScD-PFP. A single colony was selected. The PCR verified isolate was designated Strain 1-6.

Strain 1-8: Transformation of Strain 1-6 with Two Copies of the *Saccharomyces cerevisiae* IMA1 and Two Copies of the *Saccharomyces mikatae* MAL11-1.

Strain 1-6 was transformed with SEQ ID NO: 10. SEQ ID NO: 10 contains 5' homology to integration locus C, an expression cassette for the ScIMA1, an expression cassette for the URA3 marker, an expression cassette for the SmMAL11-1, and 3' homology to integration locus C locus. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. A single colony is selected. Presence of the integration at locus C is verified by PCR. The PCR verified isolate is saved as Strain 1-7.

Strain 1-7 was transformed with SEQ ID NO: 11. SEQ ID NO: 11 contains 5' homology to integration locus C, an expression cassette for the ScIMA1, an expression cassette for the *Aspergillus nidulans* acetamidase gene (amdS), an expression cassette for the SmMAL11-1, and 3' homology to the integration locus C. Transformants were selected on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 1 g/L acetamide as the sole nitrogen source. Resulting transformants were streaked for single colony isolation on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 1 g/L acetamide as the sole nitrogen source. A single colony was selected. Correct integration of SEQ ID NO: 11 at the remaining allele of locus C was verified by PCR. The PCR verified isolate was designated Strain 1-8.

Strain 1-9: Recycling the URA3 and amdS Markers via cre Recombinase in Strain 1-8

Strain 1-8 was transformed with SEQ ID NO: 9. SEQ ID NO: 9 contains the following elements: i) an expression cassette for a mutant version of a 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase gene from *Saccharomyces cerevisiae* (ARO4-OPF); 2) an expression cassette for a cre recombinase from P1 bacteriophage; 3) an expression cassette containing the native URA3, and 4) the *Saccharomyces cerevisiae* CEN6 centromere. Transformants were selected on synthetic complete media containing 3.5 g/L of p-fluorophenylalanine, and 1 g/L L-tyrosine (ScD-PFP). Resulting transformants were streaked for single colony isolation on ScD-PFP. A single colony was selected. The PCR verified isolate was designated Strain 1-9.

Strain 1-10: Restoring the Native URA3 at the Original Locus in Strain 1-9

Strain 1-9 was transformed with SEQ ID NO: 12. SEQ ID NO: 12 contains the follow elements: 1) an expression cassette for the native URA3, with 5' and 3' homology to the disrupted URA3 locus in Strain 1-6. Transformants were selected on ScD-ura. Resulting transformants were streaked

for single colony isolate on ScD-ura. A single colony was selected. The PCR verified isolate was designated Strain 1-10.

Strain 1-11 *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* cytochrome b2.

Strain 1-9 is co-transformed with SEQ ID NO: 13 (pGPL5) and SEQ ID NO: 14 (pGPL7). SEQ ID NO: 13 contains the following elements in order: 1) flanking sequence corresponding to the upstream region of the *S. cerevisiae* CAN1 gene 2) an expression cassette containing a TDH3 promoter, *Saccharomyces cerevisiae* cytochrome b2 (CYB2) in SEQ ID NO: 15 and CYC1 terminator, and 3) 1 oxP recombination sequence and the 5' portion of an expression cassette for an orotidine-5'-phosphate decarboxylase (URA3). SEQ ID NO: 14 contains the following elements in order: 1) the 3' portion of an expression cassette for an orotidine-5'-phosphate decarboxylase (URA3) and a 1 oxP recombination sequence, 2) a cassette containing an ADH1 promoter and GAL10 terminator, and 4) flanking sequence corresponding to the downstream region of the *S. cerevisiae* CAN1 gene. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-11.

Strain 1-12: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* Cytochrome b2.

Strain 1-9 is co-transformed with SEQ ID NO: 14 and SEQ ID NO: 16. SEQ ID NO: 16 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Issatchenkia orientalis* cytochrome b2 (SEQ ID NO: 17). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-12.

Strain 1-13: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Aspergillus niger* Lactic Oxidase.

Strain 1-9 is co-transformed with SEQ ID NO: 14 and SEQ ID NO: 18. SEQ ID NO: 18 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Aspergillus niger* lactic oxidase with the C-terminal peroxisomal targeting sequence removed (SEQ ID NO: 19). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-13.

Strain 1-14: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccha-*

romyces cerevisiae Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Yarrowia lipolytica* Lactic Oxidase.

Strain 1-9 is co-transformed with SEQ ID NO: 14 and SEQ ID NO: 20. SEQ ID NO: 20 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Yarrowia lipolytica* lactic oxidase with the C-terminal perioxosomal targeting sequence removed SEQ ID NO: 21. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-14.

Strain 1-15: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 22 (pGPL6) and SEQ ID NO: 23 (pESL4). SEQ ID NO: 22 contains the following elements in order: 1) flanking sequence corresponding to the upstream region of the *S. cerevisiae* CAN1 gene 2) a cassette containing a TDH3 promoter, and CYC1 terminator, and 3) loxP recombination sequence and the 5' portion of an expression cassette for an orotidine-5'-phosphate decarboxylase (URA3). SEQ ID NO: 23 contains the following elements in order: 1) the 3' portion of an expression cassette for an orotidine-5'-phosphate decarboxylase (URA3) and a loxP recombination sequence, 2) an expression cassette containing an ADH1 promoter, *Issatchenkia orientalis* monocarboxylate/proton symporter (JEN1) found in SEQ ID NO: 24, and a GAL10 terminator, and 4) flanking sequence corresponding to the downstream region of the *S. cerevisiae* CAN1 gene. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-15.

Strain 1-16: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 22 and SEQ ID NO: 25 (pESL1). SEQ ID NO: 25 is similar to SEQ ID NO: 23 with the following difference: the *Issatchenkia orientalis* monocarboxylate/proton symporter gene (JEN1) in SEQ ID NO: 23 is replaced with the *Saccharomyces cerevisiae* monocarboxylate/proton symporter (JEN1) gene (SEQ ID NO: 26). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-16.

Strain 1-17: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 22 and SEQ ID NO: 27 (pESL7). SEQ ID NO: 27 is similar to SEQ ID NO: 23 with the following difference: the *Issatchenkia orientalis* monocarboxylate/proton symporter gene (JEN1) in SEQ ID NO: 23 is replaced with the *Kluyveromyces lactis* monocarboxylate/proton symporter (JEN1) gene (SEQ ID NO: 28). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-17.

Strain 1-18: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* Acetate Transporter (ADY2^{Leu219Val, Ala252Gly}).

Strain 1-9 is co-transformed with SEQ ID NO: 22 and SEQ ID NO: 29 (pESL8). SEQ ID NO: 29 is similar to SEQ ID NO: 23 with the following difference: the *Issatchenkia orientalis* monocarboxylate/proton symporter gene (JEN1) in SEQ ID NO: 23 is replaced with the *Saccharomyces cerevisiae* acetate transporter (ADY2^{Leu219Val, Ala252Gly}) gene (SEQ ID NO: 30). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-18.

Strain 1-19: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Aggregatibacter actinomycetemcomitans* LDH and a *Saccharomyces cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 25 and SEQ ID NO: 31. SEQ ID NO: 31 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Aggregatibacter actinomycetemcomitans* LDH (SEQ ID NO: 32). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-19.

Strain 1-20: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Ornithorhynchus anatinus* LDH and a *Saccharomyces cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 25 and SEQ ID NO: 33. SEQ ID NO: 33 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Ornithorhynchus anatinus* LDH (SEQ ID NO: 34). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-20.

Strain 1-21: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* Cytochrome b2 and an *Issatchenkia orientalis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 16 and SEQ ID NO: 23. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-21.

Strain 1-22: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* Cytochrome b2 and a *Saccharomyces cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 16 and SEQ ID NO: 25. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-22.

Strain 1-23: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 16 and SEQ ID NO: 27. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-23.

Strain 1-24: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* Cytochrome b2 and a *Saccharomyces cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 13 and SEQ ID NO: 25. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-24.

Strain 1-25: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 13 and SEQ ID NO: 27. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by

PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-25.

Strain 1-26: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Aspergillus niger* Lactic Oxidase and a *Saccharomyces cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 18 and SEQ ID NO: 25. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-26.

Strain 1-27: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Yarrowia lipolytica* Lactic Oxidase and a *Saccharomyces cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 20 and SEQ ID NO: 25. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-27.

Strain 1-28: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Acetobacter aceti* D-Lactic Oxidase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 35 and SEQ ID NO: 27. SEQ ID NO: 35 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Acetobacter aceti* D-lactic oxidase (SEQ ID NO: 36). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-28.

Strain 1-29: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Gluconobacter oxydans* D-Lactic Oxidase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 37 and SEQ ID NO: 27. SEQ ID NO: 37 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Gluconobacter oxydans* D-lactic oxidase (SEQ ID NO: 38). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-29.

Strain 1-30: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 39 and SEQ ID NO: 27. SEQ ID NO: 39 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Saccharomyces cerevisiae* D-lactic dehydrogenase (SEQ ID NO: 40). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-30.

Strain 1-31: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* D-lactic Dehydrogenase A and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 41 and SEQ ID NO: 27. SEQ ID NO: 41 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Issatchenkia orientalis* D-lactic dehydrogenase (SEQ ID NO: 42). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-31.

Strain 1-32: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* D-Lactic Dehydrogenase B and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 43 and SEQ ID NO: 27. SEQ ID NO: 43 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Issatchenkia orientalis* D-lactic dehydrogenase (SEQ ID NO: 44). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-32.

Strain 1-33: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Issatchenkia orientalis* D-Lactic Dehydrogenase C and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 45 and SEQ ID NO: 27. SEQ ID NO: 45 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Issatchenkia orientalis* D-lactic dehydrogenase (SEQ ID NO: 46). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on

ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-33.

Strain 1-34: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, Integration Locus Control.

Strain 1-9 is co-transformed with SEQ ID NO: 14 and SEQ ID NO: 22. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-34.

Strain 1-35: *Saccharomyces cerevisiae* Expressing a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-3 is co-transformed with SEQ ID NO: 22 and SEQ ID NO: 27 (pESL7). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-35.

Strain 1-36: *Saccharomyces cerevisiae* Expressing a *Saccharomyces cerevisiae* Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-3 is co-transformed with SEQ ID NO: 13 and SEQ ID NO: 27. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-36.

Strain 1-37: *Saccharomyces cerevisiae* Expressing a *Saccharomyces cerevisiae* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-3 is co-transformed with SEQ ID NO: 40 and SEQ ID NO: 27. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-37.

Strain 1-38: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Lactobacillus fermentum* Lactate Racemase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 47 and SEQ ID NO: 27. SEQ ID NO: 47 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Lactobacillus fermentum* lactate racemase (SEQ ID NO: 48). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR.

Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-38.

Strain 1-39: *Saccharomyces cerevisiae* Expressing a *Lactobacillus fermentum* Lactate Racemase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-3 is co-transformed with SEQ ID NO: 47 and SEQ ID NO: 27. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-39.

Strain 1-40: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* Cytochrome b2 and a *Saccharomyces cerevisiae* Acetate Transporter (ADY2^{Leu219Val, Ala252Gly}).

Strain 1-9 is co-transformed with SEQ ID NO: 13 and SEQ ID NO: 29. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-25.

Strain 1-41: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and 2 copies of a *Saccharomyces cerevisiae* Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-25 was transformed with SEQ ID NO: 49 and SEQ ID NO: 50. SEQ ID NO: 49 contains the following elements in order: 1) flanking sequence corresponding to the upstream region of the *S. cerevisiae* CAN1, 2) a cassette containing a TDH3 promoter, the *Saccharomyces cerevisiae* CYB2, and CYC1 terminator, and 3) loxP recombination sequence and the 5' portion of the expression cassette for the *Aspergillus nidulans* acetamidase gene (amdS). SEQ ID NO: 50 contains the following elements in order: 1) the 3' portion of an expression cassette for the *Aspergillus nidulans* acetamidase (amdS), and a loxP recombination sequence, 2) an expression cassette containing an ADH1 promoter, *Kluyveromyces lactis* monocarboxylate/proton symporter (JEN1), and a GAL10 terminator, and 4) flanking sequence corresponding to the downstream region of the *S. cerevisiae* CAN1. Transformants were selected on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 1 g/L acetamide as the sole nitrogen source. Resulting transformants were streaked for single colony isolation on Yeast Nitrogen Base (without ammonium sulfate or amino acids) containing 1 g/L acetamide as the sole nitrogen source. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-41.

Strain 1-42: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces kluyveri* Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 53 and SEQ ID NO: 27. SEQ ID NO: 53 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Saccharomyces kluyveri* cytochrome b2 (SEQ ID NO: 54). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-42. Strain 1-43: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces bayanus* Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 55 and SEQ ID NO: 27. SEQ ID NO: 55 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Saccharomyces bayanus* cytochrome b2 (SEQ ID NO: 56). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-43. Strain 1-44: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 57 and SEQ ID NO: 27. SEQ ID NO: 57 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Zygosaccharomyces rouxii* cytochrome b2 (SEQ ID NO: 58). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-44.

Strain 1-45: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Kluyveromyces lactis* Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 59 and SEQ ID NO: 27. SEQ ID NO: 59 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Kluyveromyces lactis* cytochrome b2 (SEQ ID NO: 60). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-45. Strain 1-46: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Kluyveromyces dobzhanskii*

Cytochrome b2 and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 61 and SEQ ID NO: 27. SEQ ID NO: 61 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 gene in SEQ ID NO: 13 is replaced with the *Kluyveromyces dobzhanskii* cytochrome b2 (SEQ ID NO: 62). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-46.

Strain 1-47: *Saccharomyces cerevisiae* Expressing a modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces kluyveri* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 63 and SEQ ID NO: 27. SEQ ID NO: 63 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Saccharomyces kluyveri* D-lactic dehydrogenase (SEQ ID NO: 64). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-47.

Strain 1-48: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces bayanus* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 65 and SEQ ID NO: 27. SEQ ID NO: 65 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Saccharomyces bayanus* D-lactic dehydrogenase (SEQ ID NO: 66). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-48.

Strain 1-49: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and an *Aspergillus fumigatus* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 67 and SEQ ID NO: 27. SEQ ID NO: 67 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Aspergillus fumigatus* D-lactic dehydrogenase (SEQ ID NO: 68). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR.

Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-49.

Strain 1-50: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Kluyveromyces lactis* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 69 and SEQ ID NO: 27. SEQ ID NO: 69 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Kluyveromyces lactis* D-lactic dehydrogenase (SEQ ID NO: 70). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-50.

Strain 1-51: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Kluyveromyces dobzhanskii* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 71 and SEQ ID NO: 27. SEQ ID NO: 71 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Kluyveromyces dobzhanskii* D-lactic dehydrogenase (SEQ ID NO: 72). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-51.

Strain 1-52: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Kluyveromyces marxianus* D-Lactic Dehydrogenase and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 73 and SEQ ID NO: 27. SEQ ID NO: 73 is similar to SEQ ID NO: 13 with the following difference: the *Saccharomyces cerevisiae* cytochrome b2 in SEQ ID NO: 13 is replaced with the *Kluyveromyces marxianus* D-lactic dehydrogenase (SEQ ID NO: 74). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-52.

Strain 1-53: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* D-Lactic Dehydrogenase, a *Saccharomyces cerevisiae* Cytochrome b2, and a *Kluyveromyces dobzhanskii* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 75. SEQ ID NO: 80 contains the following elements in order: 1) flanking sequence corresponding to the

upstream region of the *S. cerevisiae* CAN1 2) an expression cassette containing a PGK promoter, *Saccharomyces cerevisiae* D-lactic dehydrogenase (DLD1) in SEQ ID NO: 40 and RPL3 terminator 3) an expression cassette containing a TDH3 promoter, *Saccharomyces cerevisiae* cytochrome b2 (CYB2) in SEQ ID NO: 15 and CYC1 terminator, and 4) loxP recombination sequence and the 5' portion of an expression cassette for an orotidine-5'-phosphate decarboxylase (URA3). SEQ ID NO: 75 is similar to SEQ ID NO: 23 with the following difference: the *Issatchenkia orientalis* monocarboxylate/proton symporter (JEN1) in SEQ ID NO: 23 is replaced with the *Kluyveromyces dobzhanskii* monocarboxylate/proton symporter (JEN1) protein (SEQ ID NO: 76). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-53.

Strain 1-54: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* D-Lactic Dehydrogenase, a *Saccharomyces cerevisiae* Cytochrome b2, and a *Kluyveromyces marxianus* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 77. SEQ ID NO: 77 is similar to SEQ ID NO: 23 with the following difference: the *Issatchenkia orientalis* monocarboxylate/proton symporter (JEN1) in SEQ ID NO: 23 is replaced with the *Kluyveromyces marxianus* monocarboxylate/proton symporter (JEN1) (SEQ ID NO: 78). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-54.

Strain 1-55: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* D-Lactic Dehydrogenase, a *Saccharomyces cerevisiae* Cytochrome b2, and a *Yarrowia lipolytica* Monocarboxylate/Proton Symporter (JEN6).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 79. SEQ ID NO: 79 is similar to SEQ ID NO: 23 with the following difference: the *Issatchenkia orientalis* monocarboxylate/proton symporter (JEN1) in SEQ ID NO: 23 is replaced with the *Yarrowia lipolytica* monocarboxylate/proton symporter (JEN6) (SEQ ID NO: 52). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-55.

Strain 1-56: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and a *Saccharomyces cerevisiae* D-Lactic Dehydrogenase, a *Saccharomyces cerevisiae* Cytochrome b2, and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 27. Transformants are selected on ScD-Ura.

Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-56.

Strain 1-57: *Saccharomyces cerevisiae* Expressing a Modified *Saccharomycopsis fibuligera* Glucoamylase, a *Saccharomyces cerevisiae* Isomaltase and a *Saccharomyces mikatae* Maltose Transporter, and Two Copies of a *Saccharomyces cerevisiae* D-Lactic Dehydrogenase, a *Saccharomyces cerevisiae* Cytochrome b2, and a *Kluyveromyces lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-56 is co-transformed with SEQ ID NO: 81 and SEQ ID NO: 50. SEQ ID NO: 81 is similar to SEQ ID NO: 80 with the difference being that the 5' portion of the URA3 marker is replaced with the 5' portion of the amdS marker. Transformants are selected on YNB+acetamide plates. Resulting transformants are streaked for single colony isolation on YNB+acetamide plates. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-57.

Strain 1-58: *S. cerevisiae* Expressing a Modified *S. fibuligera* Glucoamylase, a *S. cerevisiae* Isomaltase and a *S. mikatae* Maltose Transporter, and a *S. cerevisiae* D-Lactic Dehydrogenase, a *S. cerevisiae* Cytochrome b2, and a *S. cerevisiae* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 25. Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-58.

Strain 1-59: *S. cerevisiae* Expressing a Modified *S. fibuligera* glucoamylase, a *S. cerevisiae* Isomaltase and a *S. mikatae* maltose Transporter, and a *S. cerevisiae* D-Lactic Dehydrogenase, a *S. cerevisiae* Cytochrome b2, and a *S. bayanus* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 82. SEQ ID NO: 82 is similar to SEQ ID NO: 50 with the following difference: the *K. lactis* JEN1 in SEQ ID NO: 50 is replaced with the *S. bayanus* JEN1 (SEQ ID NO: 83). Transformants are selected on ScD-Ura. Resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-59.

Strain 1-60: *S. cerevisiae* Expressing a Modified *S. fibuligera* Glucoamylase, a *S. cerevisiae* Isomaltase and a *S. mikatae* maltose Transporter, and a *S. cerevisiae* D-Lactic Dehydrogenase, a *S. cerevisiae* Cytochrome b2, and a *K. lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 84. SEQ ID NO: 84 is similar to SEQ ID NO: 27 with the difference being that the ADH1 promoter SEQ ID NO: 27 is replaced with the GPD1 promoter. Transformants are selected on ScD-Ura, and the resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR.

Three independent transformants that were confirmed by PCR to contain a single copy of the expression cassette, were transformed for the second copy of the expression cassette using SEQ ID NO: 81 and SEQ ID NO: 87. SEQ ID NO: 87 is similar to SEQ ID NO: 50 with the difference being that the ADH1 promoter in SEQ ID NO: 50 is replaced with the GPD1 promoter. Transformants are selected on YNB+acetamide plates. Resulting transformants are streaked for single colony isolation on YNB+acetamide plates. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-60.

Strain 1-61: *S. cerevisiae* Expressing a Modified *S. fibuligera* Glucoamylase, a *S. cerevisiae* Isomaltase and a *S. mikatae* Maltose Transporter, and a *S. cerevisiae* D-Lactic Dehydrogenase, a *S. cerevisiae* Cytochrome b2, and a *K. lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 85. SEQ ID NO: 85 is similar to SEQ ID NO: 27 with the difference being that the ADH1 promoter SEQ ID NO: 27 is replaced with the ADH2 promoter. Transformants are selected on ScD-Ura, and the resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR.

Three independent transformants that were confirmed by PCR to contain a single copy of the expression cassette, were transformed for the second copy of the expression cassette using SEQ ID NO: 81 and SEQ ID NO: 88. SEQ ID NO: 88 is similar to SEQ ID NO: 50 with the difference being that the ADH1 promoter in SEQ ID NO: 50 is replaced with the ADH2 promoter. Transformants are selected on YNB+acetamide plates. Resulting transformants are streaked for single colony isolation on YNB+acetamide plates. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-61.

Strain 1-62: *S. cerevisiae* Expressing a Modified *S. fibuligera* Glucoamylase, a *S. cerevisiae* Isomaltase and a *S. mikatae* maltose Transporter, and a *S. cerevisiae* D-Lactic Dehydrogenase, a *S. cerevisiae* Cytochrome b2, and a *K. lactis* Monocarboxylate/Proton Symporter (JEN1).

Strain 1-9 is co-transformed with SEQ ID NO: 80 and SEQ ID NO: 86. SEQ ID NO: 86 is similar to SEQ ID NO: 27 with the difference being that the ADH1 promoter SEQ ID NO: 27 is replaced with the PDC1 promoter. Transformants are selected on ScD-Ura, and the resulting transformants are streaked for single colony isolation on ScD-Ura. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR.

Three independent transformants that were confirmed by PCR to contain a single copy of the expression cassette, were transformed for the second copy of the expression cassette using SEQ ID NO: 81 and SEQ ID NO: 89. SEQ ID NO: 89 is similar to SEQ ID NO: 50 with the difference being that the ADH1 promoter in SEQ ID NO: 50 is replaced with the PDC1 promoter. Transformants are selected on YNB+acetamide plates. Resulting transformants are streaked for single colony isolation on YNB+acetamide plates. Single colonies are selected, and the correct integration of the expression cassette is confirmed by PCR. Three

independent transformants are tested in a shake flask fermentation and a representative isolate is designated Strain 1-62.

TABLE 1

Summary Descriptions of Engineered Yeast		
Strain	Parent	Description
10	Strain 1	N/A <i>Saccharomyces cerevisiae</i> (Lasaffre, Ethanol Red)
	Strain 1-1	Strain ura3Δ/URA3, ARO4-OPF+ 1
	Strain 1-2	Strain ura3Δ, ARO4-OPF+, amdS+ 1-1
	Strain 1-3	Strain ura3Δ 1-2
15	Strain 1-4	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; URA3+, 1-3
	Strain 1-5	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; URA3+; amdS+ 1-4
	Strain 1-6	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; ura3- 1-5
20	Strain 1-7	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; URA3+ 1-6
	Strain 1-8	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; URA3+, amdS+ 1-7
25	Strain 1-9	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; ura3- 1-8
	Strain 1-10	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; URA3+ 1-9
30	Strain 1-11	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> CYB2+; URA3+ 1-9
	Strain 1-12	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> CYB2+; URA3+ 1-9
35	Strain 1-13	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Aspergillus niger</i> L-LOX+; URA3+ 1-9
	Strain 1-14	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Yarrowia lipolytica</i> L-LOX+; URA3+ 1-9
40	Strain 1-15	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> JEN1+; URA3+ 1-9
	Strain 1-16	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> JEN1+; URA3+ 1-9
45	Strain 1-17	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Kluyveromyces lactis</i> JEN1+; URA3+ 1-9
	Strain 1-18	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> ADY2 (ADY2Leu219Val, Ala252Gly)+; URA3+ 1-9
50	Strain 1-19	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Aggregatibacter actinomycetemcomitans</i> LDH, <i>Saccharomyces cerevisiae</i> JEN1+; URA3+ 1-9
	Strain 1-20	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Omithorhynchus anatinus</i> LDH, <i>Saccharomyces cerevisiae</i> JEN1+; URA3+ 1-9
55	Strain 1-21	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> CYB2+, <i>Issatchenkia orientalis</i> JEN1+; URA3+ 1-9
60	Strain 1-22	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> CYB2+, <i>Saccharomyces cerevisiae</i> JEN1+; URA3+ 1-9
65	Strain 1-23	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+ 1-9

TABLE 1-continued

Summary Descriptions of Engineered Yeast		
Strain	Parent	Description
Strain 1-24	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> CYB2+, <i>Saccharomyces cerevisiae</i> JEN1+; URA3+
Strain 1-25	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-26	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Aspergillus niger</i> L-LOX, <i>Saccharomyces cerevisiae</i> JEN1+; URA3+
Strain 1-27	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Yarrowia lipolytica</i> L-LOX, <i>Saccharomyces cerevisiae</i> JEN1+; URA3+
Strain 1-28	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>A. acetii</i> D-LOX+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-29	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Gluconobacter oxydans</i> D-LOX+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-30	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-31	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> DLD1a+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-32	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> DLD1b+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-33	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Issatchenkia orientalis</i> DLD1c+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-34	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; CAN1 site control; URA3+
Strain 1-35	Strain 1-3	ura3Δ; <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-36	Strain 1-3	ura3Δ; <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-37	Strain 1-3	ura3Δ; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-38	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+; <i>Saccharomyces mikatae</i> MAL11+; <i>Lactobacillus fermentum lactate racemase</i> +, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-39	Strain 1-3	ura3Δ; <i>Lactobacillus fermentum lactate racemase</i> +, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-40	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> CYB2+, <i>Saccharomyces cerevisiae</i> ADY2 (ADY2Leu219Val, Ala252Gly)+; URA3+
Strain 1-41	Strain 1-25	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; 2x <i>Saccharomyces cerevisiae</i> CYB2+, 2x <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-42	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces kluyveri</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-43	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces bayanus</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-44	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Zygosaccharomyces rouxii</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+

TABLE 1-continued

Summary Descriptions of Engineered Yeast		
Strain	Parent	Description
Strain 1-45	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Kluyveromyces lactis</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-46	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Kluyveromyces dobzhanskii</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-47	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+; <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces kluyveri</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-48	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces bayanus</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-49	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Aspergillus fumigatus</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-50	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Kluyveromyces lactis</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-51	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Kluyveromyces dobzhanskii</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-52	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Kluyveromyces marxianus</i> DLD1+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-53	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces dobzhanskii</i> JEN1+; URA3+
Strain 1-54	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces marxianus</i> JEN1+; URA3+
Strain 1-55	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Yarrowia lipolytica</i> JEN6+; URA3+
Strain 1-56	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-57	Strain 1-56	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-58	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Saccharomyces cerevisiae</i> JEN1+; URA3+
Strain 1-59	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Saccharomyces bayanus</i> JEN1+; URA3+
Strain 1-60	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+
Strain 1-61	Strain 1-9	<i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DLD1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+

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TABLE 1-continued

Summary Descriptions of Engineered Yeast	
Strain	Parent Description
Strain 1-62	Strain <i>Saccharomycopsis fibuligera</i> GLA1+; <i>Saccharomyces cerevisiae</i> IMA1+, <i>Saccharomyces mikatae</i> MAL11+; <i>Saccharomyces cerevisiae</i> DL.D1+, <i>Saccharomyces cerevisiae</i> CYB2+, <i>Kluyveromyces lactis</i> JEN1+; URA3+

Example 2

Evaluation of L-Lactate Consumption in Engineered *Saccharomyces cerevisiae* Strains Using an Ethanol Fermentation Shake Flask Assay

Described below are evaluations and associated data for ethanol fermentations run using selected yeast strains from Example 1. Strains 1 and 1-10 are reference strains used as comparators for various engineered strains. Strain 1 is a *Saccharomyces cerevisiae* yeast having commercially suitable ethanol tolerance. Strain 1-10 is a strain derived from strain 1 that is also suitable for commercial ethanol production.

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Additionally, the media is supplemented with sodium L-lactate (Sigma; catalog number L7022). The pH of the media is adjusted to pH 4.5, and sterile filtered prior to use.

The inoculated flask is incubated at 30° C. with shaking in an orbital shake at 75 rpm for at least 40 hours. Samples are taken and analyzed for gluconse, ethanol and lactate concentration in the broth during fermentation using high performance liquid chromatography with a refractive index detector.

Example 2A

Evaluation of Overexpression of CYB2 in Strain 1-9

As shown in Table 2 below, a strain having a native CYB2 knocked-out (strain 1-10) does not appear to consume any L-lactate. The increase in L-lactate may be due to evaporation of water during the fermentation, resulting in a slight concentration increase. However, strains containing an over-expressed CYB2 (strains 1-11 and 1-12) perform similarly to a strain with a native CYB2 (Strain 1). Specifically, strains with a CYB2 consume very small amounts of L-Lactate while producing commercially significant ethanol amounts primarily by consuming gluconse.

TABLE 2

L-Lactic acid consumption in a fermentation shake flask assay for <i>Saccharomyces cerevisiae</i> strains, including strains containing an overexpressed CYB2.									
Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (23 hours)			Final Concentration (g/L) (48 hours)		
	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate
Strain 1	256.1	0	2.9	57.4	84.6	2.8	0.9	110.7	2.5
Strain 1-10	256.1	0	2.9	57.7	84.4	3	1	111.8	3.1
Strain 1-11	256.1	0	2.9	56.5	85.7	2.7	0.6	111.5	2.5
Strain 1-12	256.1	0	2.9	61.4	83.2	2.7	1.3	111.7	2.5

Shake Flask Evaluation using Defined Media.

Selected strains from those listed in Table 1 are streaked out on a ScD-Ura plate and incubated at 30° C. until single colonies are visible (1-2 days) or grown overnight in 4 ml of YP+100 g/L D-gluconse in a 14 ml culture tube as a liquid culture at 30° C./250 rpm to generate seed biomass. A shake flask is inoculated with seed cell slurry to reach an initial OD₆₀₀ of 0.1-0.3 immediately prior to inoculating. 60 mL of shake flask medium is added to a 250 mL non-baffled shake flask (Corning 4995-250) fitted with a screw cap containing a gas-permeable seal (Corning 1395-45LTMC). The shake flask medium is prepared according to Verduyn (Verduyn, et al., 1992, Yeast. Vol. 8, 501-517), with the exception of substituting manganese tetrahydrate with manganese dihydrate. The medium is supplemented with D-gluconse to target an initial D-gluconse concentration of between 250 grams to 300 grams of D-gluconse per liter of media.

Example 2B

Evaluation of Overexpression of CYB2 and *S. cerevisiae* JEN1 in Strain 1-9

As shown in Table 3 below, strains having both an overexpressed CYB2 and an overexpressed *S. cerevisiae* JEN1 (strains 1-22 and 1-24) show significant L-lactate consumption with a high amount of gluconse still present in the fermentation broth. However, a strain having native CYB2 and JEN1 (strain 1) consumes L-lactate mostly only after the gluconse concentration is very low, while strains having no CYB2 (strains 1-10, 1-16, and 1-34) do not consume L-lactate. FIGS. 1A-1C show data for strain 1-24 corresponding to a second fermentation shake flask run.

TABLE 3

L-lactate consumption in fermentation shake flask assays for <i>Saccharomyces cerevisiae</i> strains, including strains containing an overexpressed CYB2 and <i>S. cerevisiae</i> JEN1.									
Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (20 hours)			Final Concentration (g/L) (41 hours)		
	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate
Strain 1	254.9	0	5.2	111	60.6	5.1	0.2	110	4.4
Strain 1-10	254.9	0	5.2	110.3	61.2	5.3	0.3	111	5.4
Strain 1-16	254.9	0	5.2	123.2	53.1	5.2	0.4	105.7	5.4
Strain 1-22	254.9	0	5.2	100.9	63.5	4.8	0	110	4.5
Strain 1-24	254.9	0	5.2	98.4	63.5	4.7	0	110.2	4.3
Strain 1-34	254.9	0	5.2	111.5	59.6	5.2	0	109.7	5.3

Example 2C

Evaluation of Overexpression of CYB2 and
Inclusion of K1 JEN1 and ScADY2 in Strain 1-9 25

As shown in Table 4 below, a strain having a native CYB2, JEN1, and ADY2 shows minimal L-lactate consumption in the presence of gluconse. Strain having an overexpressed CYB2 and either an overexpressed JEN1 (strain 1-24) or an ADY2 with mutations (strain 1-40) show significantly higher L-lactate consumption in the presence of gluconse. A strain having an overexpressed CYB2 and a *K. lactis* JEN1 (strain 1-25) has the highest L-lactate consumption. Further, strains 1-24, 1-25, and 1-40 show significantly higher ethanol titer than strain 1.

TABLE 4

L-lactate consumption in fermentation shake flask assays for <i>Saccharomyces cerevisiae</i> strains, including strains containing an overexpressed CYB2 and either a <i>Klactis</i> JEN1 or a ScADY2 with mutations.											
Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (20 hours)				Final Concentration (g/L) (41 hours)			
	Glucose	EtOH	L-Lactate	Glucose	EtOH	L-Lactate	L-Lactate consumption rate (gL ⁻¹ h ⁻¹) 20 h	Glucose	EtOH	L-Lactate	L-Lactate consumption rate (gL ⁻¹ h ⁻¹) 41 h
Strain 1	308.5	0	5.7	209.3	60.6	5.1	0.029	52.1	109.4	5.3	0.010
Strain 1-10	308.5	0	5.7	202.6	61.2	5.3	0.020	40.6	114.1	6.1	-0.010
Strain 1-24	308.5	0	5.7	202.1	63.5	4.8	0.044	37.9	116	4.7	0.024
Strain 1-25	308.5	0	5.7	199.2	63.5	4.7	0.049	36.7	118	4	0.041
Strain 1-40	308.5	0	5.7	199.2	43.6	5.7	0.000	30.3	120.5	4.7	0.024

Evaluation of Overexpression of Lactic Oxidase in Strain 1-9

Strains with an overexpressed lactic oxidase (L-LOX) might be expected to show improved L-lactate consumption. However, as shown in Table 5 below, strains having an overexpressed L-LOX (strains 1-13 and 1-14) do not show improved L-lactate consumption versus the comparator strains.

TABLE 5

L-lactate consumption in *Saccharomyces cerevisiae* strains, including strains containing an overexpressed lactic oxidase in fermentation shake flask assays.

Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (20 hours)			Final Concentration (g/L) (41 hours)		
	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate
Strain 1	254.9	0	5.2	111	60.6	5.1	0.2	110	4.4
Strain 1-10	254.9	0	5.2	110.3	61.2	5.3	0.3	111	5.4
Strain 1-13	254.9	0	5.2	112.1	59.5	5.2	0.2	109.8	5.3
Strain 1-14	254.9	0	5.2	104.7	62.1	5.2	0	110.9	5.3
Strain 1-34	254.9	0	5.2	111.5	59.6	5.2	0	109.7	5.3

Example 2E

Evaluation of Overexpression of Lactic Oxidase and an Overexpressed JEN1 in Strain 1-9

As discussed above, strains with an overexpressed lactic oxidase (L-LOX) might be expected to show improved L-lactate consumption, but do not show improved L-lactate consumption. Further, as shown in Table 6 below, strains having an overexpressed JEN1 (strains 1-26 and 1-27) and a L-LOX also do not show improved L-lactate consumption.

In addition, a strain having an *I. orientalis* CYB2 and an *I. orientalis* JEN1 (strain 1-21) exhibits the same L-lactate consumption as strain 1, but with lower ethanol production and gluconase consumption, even though the parent wild-type organism (*I. orientalis*) can grow on L-lactate as a carbon source.

TABLE 6

L-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* strains, including strains containing an overexpressed lactic oxidase and an overexpressed JEN1, and a strain having *I. orientalis* CYB2 and *I. orientalis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (41.3 hours)		
	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate
Strain 1	307.1	0	5.5	17.9	120.5	4.5
Strain 1-10	307.1	0	5.5	25	116.8	5.5
Strain 1-16	307.1	0	5.5	53.7	99.7	5.4
Strain 1-21	307.1	0	5.5	28.8	112.9	4.5

TABLE 6-continued

L-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* strains, including strains containing an overexpressed lactic oxidase and an overexpressed JEN1, and a strain having *I. orientalis* CYB2 and *I. orientalis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (41.3 hours)		
	Glucose	Ethanol	L-Lactate	Glucose	Ethanol	L-Lactate
Strain 1-26	307.1	0	5.5	20.4	116.5	5.5
Strain 1-27	307.1	0	5.5	20.2	114.8	5.6

Example 2F

Evaluation of Overexpression of a Lactate Dehydrogenase in Combination with a Sc JEN1 in Strain 1-9

Strains including an overexpressed lactate dehydrogenase might be expected to show improved L-lactate consumption, especially if anaerobic fermentation is expected. However, Table 7 shows that strains including a lactate dehydrogenase (LDH) (strains 1-19 and 1-20) actually produce L-lactate instead of consuming L-lactate.

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TABLE 7

L-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* strains, including strains containing an overexpressed lactate dehydrogenase and JEN1 transporter.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (41.3 hours)		
	Glucose	Ethanol	L- Lactate	Glucose	Ethanol	L- Lactate
Strain 1	307.1	0	5.5	17.9	120.5	4.5
Strain 1-10	307.1	0	5.5	25.0	116.8	5.5
Strain 1-19	307.1	0	5.5	29.0	106.4	15.4
Strain 1-20	307.1	0	5.5	100.7	67.7	33.3

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TABLE 7-continued

L-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* strains, including strains containing an overexpressed lactate dehydrogenase and JEN1 transporter.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (41.3 hours)		
	Glucose	Ethanol	L- Lactate	Glucose	Ethanol	L- Lactate
Strain 1-16	307.1	0	5.5	53.7	99.7	5.4

Example 2G

Evaluation of Various Heterologous CYB2 Genes with *K. lactis* JEN1 in Strain 1-9

As shown in Table 8, a strain containing a *Z. rouxii* CYB2 (strain 1-44) or a *K. lactis* CYB2 (strain 1-45) showed higher L-lactate consumption and higher ethanol titer than the strain with a Sc CYB2. Strains with other CYB2 (1-42, 1-43, and 1-46) showed significant L-lactate consumption and commercially relevant ethanol titers.

TABLE 8

L-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* strains, including strains containing an overexpressed CYB2 and *K. lactis* JEN1

Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (19.5 hours)			Final Concentration (g/L) (40 hours)		
	Glucose	Ethanol	L- Lactate	Glucose	Ethanol	L- Lactate	Glucose	Ethanol	L- Lactate
Strain 1	282.0	0	5.5	157.3	53.8	5.5	2.3	126.6	4.7
Strain 1-10	282.0	0	5.5	165.0	53.9	5.8	9.9	122.5	5.7
Strain 1-17	282.0	0	5.5	149.7	59.4	5.2	8.0	125.1	5.6
Strain 1-25	282.0	0	5.5	144.3	59.8	5.0	0.6	128.2	3.9
Strain 1-42	282.0	0	5.5	151.3	58.8	4.5	4.6	126.8	3.1
Strain 1-43	282.0	0	5.5	147.4	57.8	5.1	6.5	126.8	4.1
Strain 1-44	282.0	0	5.5	143.1	59.5	4.6	2.0	129.0	3.0
Strain 1-45	282.0	0	5.5	149.3	57.1	4.4	2.4	129.1	3.0
Strain 1-46	282.0	0	5.5	151.3	58.3	4.6	6.6	126.7	3.5

Evaluation of Various Heterologous
Monocarboxylate/Proton Symporters in Strain 1-9

As shown in Table 9, two strains with *Kluyveromyces* JEN1 (*K. dobzhanskii*, strain 1-53; *K. lactis*, strain 1-56) showed the highest lactate consumption and ethanol titers. The strains including a *Kluyveromyces marxianus* JEN1 (strain 1-54) and *Yarrowia lipolytica* JEN6 (strain 1-55) also showed improved lactate consumption compared to reference strains.

TABLE 9

Total lactate consumption (mixture of L- and D-lactate) in fermentation shake flask assays for <i>S. cerevisiae</i> strains, including strains containing an overexpressed <i>S. cerevisiae</i> DLD1, <i>S. cerevisiae</i> CYB2, and JEN1 or JEN6.									
Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (19.5 hours)			Final Concentration (g/L) (40 hours)		
	Glucose	Ethanol	Total Lactate	Glucose	Ethanol	Total Lactate	Glucose	Ethanol	Total Lactate
Strain 1	275.9	0.0	8.1	159.2	47.3	7.8	4.8	120.9	7.0
Strain 1-10	275.9	0.0	8.1	158.6	47.8	7.9	8.0	120.4	7.7
Strain 1-53	275.9	0.0	8.1	155.6	50.6	7.1	7.5	121.5	5.4
Strain 1-54	275.9	0.0	8.1	155.7	49.3	7.2	6.7	118.6	6.0
Strain 1-55	275.9	0.0	8.1	180.2	40.7	7.4	19.0	115.5	6.3
Strain 1-56	275.9	0.0	8.1	155.0	50.2	7.2	3.9	122.8	4.7

Example 3

Evaluation of D-Lactate Consumption in
Engineered *Saccharomyces cerevisiae* Strains Using
an Ethanol Fermentation Shake Flask Assay

Described below are evaluations and associated data for ethanol fermentations run using selected yeast strains from Example 1. Shake flask evaluation is performed as described in Example 2, except sodium D-lactate (Sigma; catalog number 71716) is used instead of sodium L-Lactate.

Example 3A

Evaluation of Overexpression of a JEN1
Transporter in Strain 1-9

As shown in Table 10 and FIGS. 2A-2C, a strain containing an overexpressed *K. lactis* JEN1 had significantly higher D-lactate consumption and also significantly higher ethanol titer than the reference strains (strains 1 and 1-10) or strains containing other overexpressed JEN1 genes (strains 1-15 and 1-16).

TABLE 10

D-lactate consumption in <i>Saccharomyces cerevisiae</i> strains, including strains containing an overexpressed JEN1 in fermentation shake flask assays.												
Strain	Starting Concentration (g/L) (0 hours)			Mid-point Concentration (g/L) (20.5 hours)			Final Concentration (g/L) (42 hours)					
	Glucose	EtOH	D-lactate	Glucose	EtOH	D-lactate	D-Lactate Consumption rate gL ⁻¹ h ⁻¹ 20.5 h		D-Lactate Consumption rate gL ⁻¹ h ⁻¹ 42 h			
Strain 1	309.1	0	3.6	169.8	51.1	3.5	0.005		15.5	117.9	3.0	0.014
Strain 1-10	309.1	0	3.6	167.9	54.6	3.5	0.005		9.2	125.8	3.1	0.012
Strain 1-15	309.1	0	3.6	165.2	52.9	3.4	0.010		29.8	116.8	2.9	0.017

TABLE 10-continued

D-lactate consumption in *Saccharomyces cerevisiae* strains, including strains containing an overexpressed JEN1 in fermentation shake flask assays.

Strain	Starting Concentration (g/L) (0 hours)			Mid-point Concentration (g/L) (20.5 hours)				Final Concentration (g/L) (42 hours)			
	Glucose	EtOH	D-lactate	Glucose	EtOH	D-Lactate	D-Lactate Consumption rate gL ⁻¹ h ⁻¹ 20.5 h	Glucose	EtOH	D-Lactate	D-Lactate Consumption rate gL ⁻¹ h ⁻¹ 42 h
Strain 1-16	309.1	0	3.6	187.5	44.6	3.4	0.010	52.1	102.0	3.0	0.014
Strain 1-17	309.1	0	3.6	161.6	53.0	2.9	0.034	15.1	131.7	1.9	0.041

Example 3B

Evaluation of Overexpression of a *Saccharomyces cerevisiae* ADY2(*ADY2^{Leu219Val, Ala252Gly}*) Permease in Strain 1-9

As shown in Table 11, D-lactate consumption and ethanol production are significantly higher in a strain containing a *K. lactis* JEN1 than in the reference strains, while a strain containing ADY2 (strain 1-18) shows performance similar to the reference strains.

TABLE 11

D-lactate consumption in *Saccharomyces cerevisiae* strains containing an overexpressed JEN1 in fermentation shake flask assays.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (42 hours)		
	Glucose	Ethanol	D-lactate	Glucose	Ethanol	Lactate
Strain 1	307.0	0	3.5	41.5	114.1	3.1
Strain 1-10	307.0	0	3.6	32.6	119.2	3.0
Strain 1-17	307.0	0	3.6	29.1	120.7	1.7

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TABLE 11-continued

D-lactate consumption in *Saccharomyces cerevisiae* strains containing an overexpressed JEN1 in fermentation shake flask assays.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (42 hours)		
	Glucose	Ethanol	D-lactate	Glucose	Ethanol	D-Lactate
Strain 1-18	307.0	0	3.6	44.5	115.0	3.1

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Example 3C

Evaluation of Overexpression of a D-Lactate Dehydrogenase and a *K. lactis* JEN1 in Strain 1-9

As shown in FIGS. 3A-3C, a strain containing a *K. Lactis* JEN1 and an overexpressed DLD (strain 1-30) shows significantly improved D-lactate consumption and a higher ethanol titer than the reference strains. Further, the D-lactate consumption of strain 1-30 is significantly improved over a strain having only the *K. Lactis* JEN1 (strain 1-17).

Example 3D

Evaluation of *Issatchenkia orientalis* D-Lactate Dehydrogenase and *K. lactis* JEN1 in Strain 1-9

Table 12 below shows data for three homolog strains containing a *K. Lactis* JEN1 and an overexpressed *I. orientalis* DLD (strains 1-31, 1-32, and 1-33). Each of these three homologs shows improved D-lactate consumption compared to a reference strain (1-10).

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TABLE 12

D-lactate consumption in fermentation shake flask assays of *Saccharomyces cerevisiae* strains containing an overexpressed D-lactate oxidase and a *K. lactis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Mid-point Concentration (g/L) (20.5 hours)			Final Concentration (g/L) (42 hours)		
	Glucose	Ethanol	D-lactate	Glucose	Ethanol	D-Lactate	Glucose	Ethanol	Lactate
Strain 1-10	301.3	0	3.9	191.8	42.3	3.7	29.9	117.8	3.3
Strain 1-31	301.3	0	3.9	194.7	41.6	2.9	37.4	114.9	1.3

TABLE 12-continued

D-lactate consumption in fermentation shake flask assays of *Saccharomyces cerevisiae* strains containing an overexpressed D-lactate oxidase and a *K. lactis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Mid-point Concentration (g/L) (20.5 hours)			Final Concentration (g/L) (42 hours)		
	Glucose	Ethanol	D-lactate	Glucose	Ethanol	D-Lactate	Glucose	Ethanol	Lactate
Strain 1-32	301.3	0	3.9	190.8	42.9	3.3	29.3	119.3	2.1
Strain 1-33	301.3	0	3.9	194.2	41.6	2.9	34.7	113.4	1.0

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Example 3E

Evaluation of Overexpressed D-Lactate Oxidase and *K. lactis* JEN1 in Strain 1-9

As shown in Table 13 below, strains containing an overexpressed D-lactate oxidase (D-LOX) and a *K. lactis* JEN1 (strains 1-28 and 1-29) show improved D-lactate consumption compared to reference strains (strains 1 and 1-10), but show less D-lactate consumption than a strain containing only a *K. lactis* JEN1 (strain 1-17). A strain containing a D-LOX might be expected to show improved D-lactate consumption, however, as seen with the L-LOX strains described above, the inclusion of D-LOX does not improve the D-lactate consumption.

TABLE 13

D-lactate consumption in fermentation shake flask assays of *Saccharomyces cerevisiae* strains containing an overexpressed D-lactate oxidase and a *K. lactis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (42 hours)		
	Glucose	Ethanol	D-lactate	Glucose	Ethanol	D-Lactate
Strain 1	307.0	0	3.5	41.5	114.1	3.1
Strain 1-10	307.0	0	3.6	32.6	119.2	3.0

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TABLE 13-continued

D-lactate consumption in fermentation shake flask assays of *Saccharomyces cerevisiae* strains containing an overexpressed D-lactate oxidase and a *K. lactis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Final Concentration (g/L) (42 hours)		
	Glucose	Ethanol	D-lactate	Glucose	Ethanol	D-Lactate
Strain 1-17	307.0	0	3.6	29.1	120.7	1.7
Strain 1-28	307.0	0	3.6	94.3	97.9	2.6
Strain 1-29	307.0	0	3.6	43.0	116.3	2.0

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Example 3F

Evaluation of Various Heterologous DLD Genes with *K. lactis* JEN1 in Strain 1-9

As shown in Table 14, a strain containing a *K. lactis* DLD (1-50) had the highest ethanol titer and L-lactate consumption than other strains with selected DLDs. However, the other strains (1-47, 1-48, 1-49, 1-51, and 1-52) all showed significant D-lactate consumption and commercially relevant ethanol titers.

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TABLE 14

D-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* strains, including strains containing an overexpressed DLD1 and *K. lactis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (19.5 hours)			Final Concentration (g/L) (40 hours)		
	Glucose	Ethanol	D-Lactate	Glucose	Ethanol	Lactate	Glucose	Ethanol	Lactate
Strain 1	282.7	0.0	4.1	146.2	59.0	4.0	1.1	127.5	3.5
Strain 1-10	282.7	0.0	4.1	140.4	59.9	3.9	1.8	127.4	3.5
Strain 1-17	282.7	0.0	4.1	140.6	61.5	3.6	1.9	128.6	2.5
Strain 1-30	282.7	0.0	4.1	135.5	64.1	3.0	4.2	127.1	1.7

D-lactate consumption in fermentation shake flask assays for *Saccharomyces cerevisiae* strains, including strains containing an overexpressed DLD1 and *K. lactis* JEN1.

Strain	Starting Concentration (g/L) (0 hours)			Mid-Point Concentration (g/L) (19.5 hours)			Final Concentration (g/L) (40 hours)		
	Glucose	Ethanol	D- Lactate	Glucose	Ethanol	D- Lactate	Glucose	Ethanol	D- Lactate
Strain 1-47	282.7	0.0	4.1	140.9	60.9	3.1	3.1	127.1	1.8
Strain 1-48	282.7	0.0	4.1	138.3	62.3	3.5	2.7	128.0	2.8
Strain 1-49	282.7	0.0	4.1	146.4	60.5	3.5	5.8	125.2	2.6
Strain 1-50	282.7	0.0	4.1	139.4	62.1	3.0	1.3	129.3	1.7
Strain 1-51	282.7	0.0	4.1	164.7	50.9	3.2	11.3	122.2	2.0
Strain 1-52	282.7	0.0	4.1	154.4	55.6	3.2	2.8	125.3	2.4

Example 4

Evaluation of a Genetically Modified *Saccharomyces cerevisiae* Strain in a Simultaneous Saccharification Fermentation (SSF) Shake Flask Assay

A *Saccharomyces cerevisiae* strain containing an overexpressed *S. cerevisiae* CYB2 and a *Kluyvermyces lactis* JEN1 is evaluated for consumption of lactic acid and D-gluconse, and also production of ethanol. Strains obtained as described in Example 1.

Strains are struck to a ScD-ura plate and incubated at 25° C. until single colonies are visible (2-3 days). Cells from the ScD-ura plate are scraped into sterile shake flask medium and the optical density (OD₆₀₀) is measured. Optical density is measured at wavelength of 600 nm with a 1 cm path length using a model Genesys20 spectrophotometer (Thermo Scientific). A shake flask is inoculated with the cell slurry to reach an initial OD₆₀₀ of 0.1. Immediately prior to inoculating, 50 mL of shake flask medium is added to a 250 mL non-baffled shake flask (Corning 4995-250) fitted with a screw cap containing a gas-permeable seal (coming 1395-45LTMC). The shake flask medium consists of 850 g partially hydrolyzed corn starch, 150 g filtered light steep water, 10 g water, 25 g gluconse, and 1 g urea. Duplicate flasks for each strain are incubated at 30° C. with shaking in an orbital shaker at 100 rpm for 48 hours. Samples are taken and analyzed for metabolite concentrations in the broth during fermentation by HPLC with refractive index and UPLC with ELSD detection. Lactic acid (both D- and L-) is a component of the light steep water and is produced through contamination of the light steep water by lactic acid producing bacteria. The fraction of the D- and L-lactic acid in

the light steep water varies between lots, and the absolute amount is not predictable. In general, the fraction of D- and L-lactic is a 50:50 racemic mix of the two enantiomers is at a total concentration of approximately 25 grams of lactic acid per liter of light steep water. Light steep water also contains low levels of gluconse.

As shown in FIGS. 4A and 4B, a strain containing an overexpressed CYB2 and a *K. Lactis* JEN1 (strain 1-25) consumes most or all of D,L-lactate while producing a similar amount of ethanol compared to the reference strains.

Example 5

Performance of Genetically Modified Yeast Strain in SSF with Relatively Low Levels of Gluconse and Relatively High Levels of D,L-Lactate

Strain 1-41 is obtained as described in Example 1. Strain 1-41 is strain 1-9 containing 2 copies of the ScCYB2 and 2 copies of the K1JEN1. Strains are run in a shake flask fermentation according to Example 4, with the following differences: 1) the shake flask medium of this Example (Example 5) is light steep water; 2) the shake flasks are inoculated with a cell slurry to reach an initial OD₆₀₀ of 4.0; 3) the non-baffled shake flasks are fitted with a Morton cap; and 4) the flasks are incubated in an orbital shaker at 150 rpm.

As shown in Tables 15 and 16 and FIGS. 5A and 5B, the strain containing overexpression of ScCYB2 and also containing K1JEN1 (1-41) consumed significantly more D,L-lactate than the reference strains, while producing a significantly higher ethanol titer than the reference strains.

TABLE 15

Overexpression of a ScCYB2 and Kl JEN1 in Strain 1-9 in a simultaneous saccharification shake flask assay

Strain	Starting Concentration (g/L) (0 hours)			Mid-point Concentration (g/L) (7 hours)			Final Concentration (g/L) (48 hours)		
	Glucose	EtOH	Lactate	Glucose	EtOH	Lactate	Glucose	EtOH	Lactate
Strain 1	11.65	0.59	25	0	6.7	21.2	0	7.4	15.9
Strain 1-10	11.65	0.59	25	0	6.5	21.8	0	5.7	19.4
Strain 1-41	11.65	0.59	25	0.16	7.3	20.3	0	8.7	13.0

TABLE 16

Rate of total lactate consumption (D- and L- Lactate)

Strain	Total Lactate consumption rate (g L ⁻¹ h ⁻¹)		
	0 to 7 hours	7 hours to 48 hours	0 to 48 hours
Strain 1	0.543	0.129	0.190
Strain 1-10	0.457	0.059	0.117
Strain 1-41	0.671	0.178	0.250

Example 6

L-Lactate Consumption in Genetically Modified Yeast Strains with Selected Heterologous Monocarboxylic/Monocarboxylate Transporters

S. cerevisiae strains containing selected heterologous monocarboxylic/monocarboxylate transporters are evaluated for L-lactate transport/consumption. As shown previously, *K. lactis* JEN1 has a higher affinity for D-lactate transport compared to L-lactate transport. Shake flask assays are performed using defined media with only L-lactate present. Dextrose concentrations are not measured, but are expected to be about 150 g/L at 20 h.

As shown in Table 17 and FIG. 6, all heterologous monocarboxylic/monocarboxylate transporters tested exhibit improved L-lactate consumption compared to reference strains (strains 1 and 1-10).

TABLE 17

L-lactate consumption in fermentation shake flask assays for *S. cerevisiae* strains, including strains containing an overexpressed *S. cerevisiae* DLD1, *S. cerevisiae* CYB2, selected heterologous monocarboxylic/monocarboxylate transporters.

Strain	Final Concentration (g/L) (41 hours)		
	Glucose	Ethanol	L- Lactate
1	0.2	130.8	1.3
1-10	0.2	130.7	2.9

TABLE 17-continued

L-lactate consumption in fermentation shake flask assays for *S. cerevisiae* strains, including strains containing an overexpressed *S. cerevisiae* DLD1, *S. cerevisiae* CYB2, selected heterologous monocarboxylic/monocarboxylate transporters.

Strain	Final Concentration (g/L) (41 hours)		
	Glucose	Ethanol	L- Lactate
1-53	0.2	132.3	0.4
1-54	0.2	131.4	1.0
1-55	0.3	133.3	1.0
1-56	0.2	132.1	0.6
1-58	0.1	131.8	0.8
1-59	0.2	132.1	0.8

Example 7

Performance of Genetically Modified Yeast Strain in Production-Scale Ethanol Fermentation Process

A continuous fermentation process for producing ethanol is run on commercial scale using a reference strain with no lactic acid consumption pathway (strain 1-10) and (separately) a strain with an exogenous lactic consumption pathway enabled (strain 1-57) using an initial fermentation media including light steep water (LSW). Stage 1 is the propagator and Stage 2 is the pre-fermenter.

The changing concentration of D/L-lactic at the different stages reflects a number of changes to the composition of the fermentation broth due to inlet streams and dilution of the broth. Dilution levels are substantially the same for each strain at each stage. Therefore, relevant comparisons are the changing ratio of D/L-lactic for strain 1-10 vs. strain 1-57 within any given stage.

As shown in Table 18, a strain having a native CYB2 knocked-out and no exogenous lactic acid consumption pathway (strain 1-10) shows higher concentrations of both L- and D-lactic in each of Stage 1 and Stage 2, relative to a strain with the exogenous lactic consumption pathway enabled (strain 1-57).

TABLE 18

Lactate consumption by genetically modified yeast strains on production scale									
raw LSW			Stage 2						
L-			Stage 1			Total			
	lactic	D-lactic	Total Lactic	L-lactic	D-lactic	Total Lactic	L-lactic	D-lactic	Lactic
	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
Strain	14.5	9.8	24.3	7.8	4.2	12.0	4.9	2.1	6.9
1-10	±2.7	±1.6	±4.1	±0.8	±1.8	±1.6	±0.3	±0.9	±0.3
Strain	13	11.1	24.1	5.6	1.7	7.3	3.9	0.5	4.4
1-57	±3.1	±3.4	±6.5	±0.7	±0.3	±0.3	±0.5	±0.8	±0.9

Example 8

Evaluation of Promoters for Driving Expression of a JEN1 Transporter

Alternative promoters to the promoter used in strain 1-57 (ADH1) are evaluated for driving expression of a JEN1 transporter. Strains were evaluated in a simultaneous saccharification fermentation (SSF) shake flask assay. These shake flasks serve as a proxy for assessing early fermentation burden of the selected strains.

In one aspect, as shown in Table 19, strains with alternative promoters to the promoter used in strain 1-57 (i.e., strains 1-60, 1-61, and 1-62) show significantly higher

ethanol titers at the mid-point (22 h), therefore exhibiting reduced fermentation burden.

In another aspect, strain 1-57 is shown to generate significantly less glycerol with a corresponding significant increase in ethanol titer compared to all other strains. All strains with an enabled exogenous lactate-consumption pathway demonstrate lactate consumption and also the same or higher final ethanol titer compared to the reference strains. This demonstrates that strains with an enabled exogenous lactate-consumption pathway combined with selected promoters can achieve higher ethanol titers and/or the same ethanol titer at an earlier time point, either of which can provide a significant commercial advantage (i.e., increased throughput or reduced cycle time, or both).

TABLE 19

SSF shake flask assays for selected strains												
Strain	Starting Concentration (g/L) (0 hours)				Mid-Point Concentration (g/L) (22 hours)				End Point Concentration (g/L) (68 hours)			
	Glucose	EtOH	Glycerol	Total Lactate	Glucose	EtOH	Glycerol	Total Lactate	Glucose	EtOH	Glycerol	Total Lactate
Strain 1	34.4	0.2	0.1	2.4	37.6	73.8	6.6	2.5	0.6	150.4	9.3	1.5
Strain 1-10	34.4	0.2	0.1	2.4	22.1	71.7	5.6	2.8	0.0	150.3	8.6	2.2
Strain 1-57	34.4	0.2	0.1	2.4	15.8	67.7	4.3	2.0	0.0	154	7.3	0.0
Strain 1-60	34.4	0.2	0.1	2.4	18.6	73.2	5.4	2.1	0.0	152.6	8.2	0.2
Strain 1-61	34.4	0.2	0.1	2.4	23	74.2	5.7	2.5	0.0	150.3	8.6	0.8
Strain 1-62	34.4	0.2	0.1	2.4	18.4	74.3	5.1	2.0	0.0	151.6	7.8	0.0

Batch SSF with Selected Strains

Strain 1-62 is benchmarked against Strain 1-10 in a lab scale batch fermentation process. The feedstock consists of a partially saccharified starch stream with a starting percent dextrose concentration between 20-35%. The starting pH is between 3.5 and 4.5 and the operating temperature is 31° C. An average OUR of 0.66 mmol O₂/(L·h) is recorded from 20 hours elapsed fermentation time (EFT) to the end of fermentation (40 hours EFT), and the vessels are run until free dextrose concentrations are 0.2-0.5 (40 hours EFT). The data represents the average of three independent fermentation vessels per strain.

The starting dextrose concentration is cross validated using a carbohydrate column (87C) to determine the free dextrose, DP2, DP3 and DP4+ concentrations in the T0+ samples and are multiplied by their respective chemical gain factors to determine the dextrose sum. This method is cross validated using an enzymatic hydrolysis method. Dextrose sum results are averaged for each vessel.

As shown in Table 20, strain 1-62 (having an exogenous lactate-consumption pathway) demonstrates a significantly higher ethanol titer and lactate consumption, and also shows significantly lower glycerol generation, than the reference strain.

TABLE 20

4 L SSF batch fermenter data for strain 1-10 vs. strain 1-62								
	Starting Concentration (n = 3)				Average Final Titer @ 40 hours (n = 3)			
	Total Dextrose (g/Kg)	Glycerol (g/Kg)	Lactate (g/Kg)	Ethanol (g/kg)	Total Dextrose (g/Kg)	Glycerol (g/Kg)	Lactate (g/Kg)	Ethanol (g/kg)
Strain 1-10	256.6 ±2.8	2.0 ±0.3	6.6 ±0.8	0.2 ±0.0	0.6 ±0.3	10.4 ±0.5	5.6 ±0.7	111 ±1.2
Strain 1-62	254.8 ±1.5	2.0 ±0.3	6.6 ±0.8	0.2 ±0.1	0.9 ±0.5	8.8 ±0.4	4.4 ±0.7	114.4 ±0.2

Exemplary Embodiments

The embodiments in this section are designated sequentially with letters A through Z, then AA through AZ, then BA, and so on.

A. A genetically modified yeast comprising a heterologous gene encoding a monocarboxylic/monocarboxylate transporter and one or more heterologous genes encoding lactate dehydrogenase (cytochrome) (classified as EC 1.1.2.3 or 1.1.2.4), wherein the yeast is capable of consuming lactate and producing ethanol when the yeast is present in a fermentation medium comprising lactate and hexose.

B. The yeast of embodiment A, wherein the yeast has a L-lactate consumption rate of at least 0.030, 0.035, 0.040, 0.045, or 0.050 gL⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 150 g/L or greater at a sampling time of 21 h, as measured according to the Evaluation Protocol for Lactate Consumption.

C. The yeast of embodiment A, wherein the yeast has a L-lactate consumption rate of at least 0.015, 0.020, 0.025, 0.030, 0.035, or 0.040 gL⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 5 g/L or greater at a sampling time of 48 h, as measured according to the Evaluation Protocol for Lactate Consumption.

D. The yeast of embodiment A, wherein the yeast has a D-lactate consumption rate of at least 0.005, 0.006, 0.007,

0.008, 0.009, 0.010, 0.015, 0.020, 0.025, or 0.030 gL⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 150 g/L or greater at a sampling time of 21 h, as measured according to the Evaluation Protocol for Lactate Consumption.

E. The yeast of embodiment A, wherein the yeast has a D-lactate consumption rate of at least 0.015, 0.020, 0.025, 0.030, 0.035, or 0.040 gL⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 5 g/L or greater at a sampling time of 48 h, as measured according to the Evaluation Protocol for Lactate Consumption.

F. The yeast of any of embodiments A-E, wherein the D-, L-, and/or total lactate consumption rate of the yeast is greater than a yeast without a heterologous gene encoding a monocarboxylic/monocarboxylate transporter.

G. The yeast of embodiment A, wherein the yeast has a total lactate consumption rate of at least 0.550, 0.555, 0.600 or 0.650 gL⁻¹h⁻¹ between time 0 and 7 hours in a simultaneous saccharification fermentation (SSF) process.

H. The yeast of embodiment A, wherein the yeast has a total lactate consumption rate of at least 0.130, 0.140, 0.150, 0.160, or 0.170 gL⁻¹h⁻¹ between time 7 hours and 48 hours in a SSF process.

I. The yeast of embodiment A, wherein the yeast has a total lactate consumption rate of at least 0.200, 0.210, 0.220, 0.230, 0.240, or 0.250 gL⁻¹h⁻¹ between time 0 and 48 hours in a SSF process.

J. The yeast of any of embodiments G-I, wherein the total lactate consumption rate is determined according to the method for SSF shake flask assay in Example 5.

K. The yeast of any of embodiments A-J, wherein the yeast is capable of consuming D-lactate, L-lactate, or a mixture thereof.

L. The yeast of any of embodiments A-K, wherein the one or more heterologous lactate dehydrogenase (cytochrome) genes comprise an overexpressed D-lactate dehydrogenase (DLD) gene.

M. The yeast of any of embodiments A-K, wherein the one or more heterologous lactate dehydrogenase (cytochrome) genes comprise an overexpressed cytochrome b2 (CYB2) gene.

N. The yeast of any of embodiments A-K, wherein the one or more heterologous lactate dehydrogenase (cytochrome) genes comprise an overexpressed DLD gene and an overexpressed CYB2 gene.

O. The yeast of any of embodiments L-N, wherein the one or more DLD genes is from one or more of the following yeast species: *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, *Saccharomyces kluyveri*, *Saccharomyces bayanus*, *Kluyveromyces dobzhanskii*, *Kluyveromyces marxianus*, or *Kluyveromyces lactis*.

P. The yeast of any of embodiments A-O, wherein the yeast encodes for a polypeptide having an amino acid

sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequences: SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, or SEQ ID NO: 74.

Q. The yeast of any of embodiments M-N, wherein the one or more CYB2 genes is from one or more of the following yeast species: *Saccharomyces cerevisiae*, *Issatchenkia orientalis*, *Saccharomyces kluyveri*, *Saccharomyces bayanus*, *Zygosaccharomyces rouxii*, *Kluyveromyces dobzhanskii*, or *Kluyveromyces lactis*.

R. The yeast of any of embodiments A-Q, wherein the yeast encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequences: SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62.

S. The yeast of any of embodiments A-R which comprises a heterologous gene encoding a lactate racemase.

T. A genetically modified yeast comprising a heterologous gene encoding a monocarboxylate/proton symporter and a heterologous gene encoding a glucoamylase, wherein the yeast is capable of consuming lactate and producing ethanol when the yeast is present in a fermentation medium comprising lactate and hexose, starch, or a combination of hexose and starch.

U. The yeast of any of embodiments A-T, wherein the yeast encodes for a glucoamylase from *Saccharomycopsis fibuligera* or *Rhizopus oryzae*.

V. The yeast of any of embodiments A-U, wherein the yeast is of the genus *Saccharomyces*.

W. The yeast of embodiment V, wherein the yeast is of the species *Saccharomyces cerevisiae*.

X. A genetically modified yeast comprising a yeast of the species *Saccharomyces cerevisiae*, wherein the yeast comprises a heterologous gene encoding a monocarboxylate/proton symporter.

Y. The yeast of embodiment X, wherein the yeast is obtained by genetically modifying a *Saccharomyces cerevisiae* host yeast and wherein the host yeast is ETHANOL RED™ (commercially available from Lesaffre).

Z. The yeast of any of embodiments A-Y which is capable of producing ethanol at a fermentation production rate of at least 1.0 g L⁻¹ h⁻¹, 2.0 g L⁻¹ h⁻¹, 3.0 g L⁻¹ h⁻¹, 3.3 g L⁻¹ h⁻¹, or 3.75 g L⁻¹ h⁻¹.

AA. The yeast of any of embodiments A-Z which is capable of producing ethanol at a titer greater than 80 g/L, 90 g/L, 100 g/L, 110 g/L, 120 g/L, 125 g/L, 130 g/L, 135 g/L, 140 g/L, 145 g/L, 150 g/L, 155 g/L, 160 g/L, or 165 g/L.

AB. The yeast of any of embodiments A-AA (i.e., A-Z and AA) which is (a) capable of producing ethanol at a titer of greater than 90 g/L, 100 g/L, 110 g/L, 120 g/L, 130 g/L, or 140 g/L; (b) thermotolerant at temperatures in the range of 33° C. to 40° C., 33° C. to 39° C., 33° C. to 38° C., 33° C. to 37° C., 34° C. to 37° C., 35° C. to 37° C., or 36° C. to 38° C.; or both (a) and (b).

AC. The yeast of any of embodiments A-AB, wherein the heterologous gene encoding a monocarboxylic/monocarboxylate transporter is from a yeast of the genus *Kluyveromyces*.

AD. The yeast of any of embodiments A-AC, wherein the heterologous gene encoding a monocarboxylic/monocarboxylate transporter is from *Kluyveromyces lactis*.

AE. The yeast of any of embodiments A-AD, wherein the yeast encodes for a polypeptide having an amino acid

sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequences: SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, or SEQ ID NO: 78.

AF. The yeast of embodiment AE, comprising one or more of the following residues at the indicated positions in SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62: Lys349, Tyr143, Tyr254, and His373.

AG. Use of the yeast of any of embodiments A-AF for the production of ethanol or a bioproduct other than ethanol.

AH. A process for producing ethanol comprising fermenting a substrate with the yeast of any of embodiments A-AG.

AI. A continuous fermentation process for manufacturing ethanol comprising: providing a fermentation medium comprising hexose or hexose oligomers, fermenting the fermentation medium with a genetically modified yeast comprising a heterologous monocarboxylate/proton symporter (JEN1) gene, adding one or more feed streams comprising lactate to the fermentation medium, and removing at least one output stream comprising ethanol from the fermentation medium, wherein the average hexose or hexose oligomer concentration of the fermentation medium is at least 5 g/L, the volumetric oxygen uptake rate of the process is at least 0.5 mmol O₂/(L·h), and the process produces ethanol at an overall rate of at least 1.0 g L⁻¹ h⁻¹, and the yeast consumes lactate, wherein the at least one output stream contains less than 90% of the lactate added in the one or more feed streams.

AJ. The process of embodiment AI, wherein lactate concentration of one or more feed streams has an average lactate concentration of at least 1 g/L.

AK. The process of any of embodiments AI-AJ, wherein the feed stream is a vegetable process stream.

AL. The process of embodiment AK, wherein the vegetable process stream is a corn process stream or a wheat process stream.

AM. The process of any of embodiments AH-AL, wherein the ethanol titer at the end of the pre-fermenter step is in the range of 20 to 80 g/L.

AN. The process of any of embodiments AH-AM, wherein the L-lactate consumption rate in the propagator step or pre-fermenter step is at least 0.030, 0.035, 0.040, 0.045, or 0.050 gL⁻¹h⁻¹.

AO. The process of any of embodiments AH-AN, wherein the D-lactate consumption rate in the propagator step or pre-fermenter step 0.005, 0.006, 0.007, 0.008, 0.009, 0.010, 0.015, 0.020, 0.025, or 0.030 gL⁻¹h⁻¹.

AP. The process of any of embodiments AH-AO, wherein the ethanol titer of the at least one output stream is at least 90, 100, 110, 120, 130, 140, 150, or 160 g/L.

AQ. A process for manufacturing ethanol comprising: fermenting a medium using a genetically modified yeast comprising a heterologous monocarboxylate/proton symporter gene, wherein the medium comprises gluconse or gluconse oligomers at a concentration of at least 5 g/L at the start of fermentation, the lactate content of the medium is at least 1 g/L at the start of fermentation, at least 40% of the lactate is consumed at the end of fermentation, and the final ethanol titer is at least 90 g/L.

AR. The process of any of embodiments AH-AQ, having a volumetric oxygen uptake rate (OUR) of at least 0.5, 1, 2, 3, or 4 mmol O₂/(L·h).

AS. A process for manufacturing ethanol comprising: fermenting a medium using a *S. cerevisiae* yeast, wherein the medium comprises gluconse or gluconse oligomers and

lactate, the OUR is at least 0.5 mmol O₂/(L·h), the lactate content of the medium is at least 1 g/L at the start of fermentation, at least 35% of the lactate present in the medium at the start of fermentation and/or added to the medium during fermentation is consumed by the end of fermentation, and the final ethanol titer is at least 90 g/L.

AT. The process of embodiment AS, wherein the OUR is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mmol O₂/(L·h).

AU. The process of any of embodiments AS-AT, wherein the lactate content of the medium is at least 2, 3, 4, or 5 g/L at the start of fermentation.

AV. The process of any of embodiments AS-AU, wherein at least 40, 45, or 50% of the lactate present in the medium at the start of fermentation and/or added to the medium during fermentation is consumed by the end of fermentation

AW. The process of any of embodiments AH-AV, wherein the yeast is the yeast of any of embodiments A-Y.

AX. The process of any of embodiments AH-AW, wherein the lactate is L-lactate, D-lactate, or a mixture thereof.

AY. The process of any of embodiments AH-AX, wherein the pH of the fermentation medium is in the range of about 2 to 7.

AZ. The process of any of embodiments AH-AY, wherein the fermentation temperature is in the range of 25 to 45° C., 25 to 40° C., 25 to 35° C., 30 to 40° C., or 28 to 38° C.

BA. The process of any of embodiments AH-AZ, wherein the dry solids of the fermentation medium is at least 30, 40, 50, 60, 70, or 80 g/L

BB. The process of any of embodiments AH-BA, wherein the dry solids of the fermentation feed is in the range of 120 to 150 g/L.

BC. The process of any of embodiments AH-BB, wherein the ethanol titer at the end of fermentation is at least 80, 90, 100, 110, 120, 130, 135, 140, 145, 150, 155, or 160 g/liter.

BD. A method for producing a genetically modified yeast with improved lactate consumption comprising overexpressing one or more heterologous genes encoding a lactate dehydrogenase (cytochrome) (classified as EC 1.1.2.3 or 1.1.2.4) or a heterologous monocarboxylate/proton symporter, and subjecting the yeast to evolution for a characteristic, wherein the yeast is capable of consuming lactate

and producing ethanol when the yeast is present in a fermentation medium comprising lactate and gluconse.

BE. The method of embodiment BD, wherein the characteristic is increased growth rate on lactate.

BF. The process or method of any of embodiments AH-BE, wherein the yeast encodes for a polypeptide having an amino acid sequence with a sequence identity of at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% to any one of the following amino acid sequence: SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, or SEQ ID NO: 78.

BG. The process or method of embodiment BF, wherein the yeast comprises one or more of the following residues at the indicated positions in SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62: Lys349, Tyr143, Tyr254, and His373.

BH. The process, method, or yeast of any of embodiments A-BG, wherein the yeast comprises a heterologous nucleic acid regulatory sequence.

BI. The process, method, or yeast of embodiment BH, wherein the heterologous nucleic acid regulatory sequence is associated with the heterologous gene encoding a monocarboxylic/monocarboxylate transporter.

BJ. The process, method, or yeast of embodiments BH or BI, wherein the heterologous nucleic acid regulatory sequence comprises the ADH1 promoter.

BK. The process, method, or yeast of embodiments BH or BI, wherein the heterologous nucleic acid regulatory sequence comprises the ADH2 promoter, the PDC1 promoter, or the GPD1 promoter.

It is also to be understood that the elements or aspects of any of the above embodiments of the processes, methods, or compositions described above can be applied to any other embodiment, even if not explicitly stated herein.

The disclosures of each and every patent, patent application, or publication cited herein are hereby incorporated by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and variations.

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and P1 bacteriophage

<400> SEQUENCE: 3

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<210> SEQ ID NO 4

<211> LENGTH: 6376

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple separate
Saccharomyces cerevisiae sequences

<400> SEQUENCE: 4

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<210> SEQ ID NO 5

<211> LENGTH: 4632

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Saccharomycopsis fibuligera

<400> SEQUENCE: 5

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<210> SEQ ID NO 6

<211> LENGTH: 4363

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and *Saccharomycopsis fibuligera*

<400> SEQUENCE: 6

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<210> SEQ ID NO 7

<211> LENGTH: 5015

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Sequence put together from multiple organisms:
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Aspergillus nidulans

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<400> SEQUENCE: 7

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<210> SEQ ID NO 8

<211> LENGTH: 4771

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
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Aspergillus nidulans

<400> SEQUENCE: 8

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<210> SEQ ID NO 12

<211> LENGTH: 1632

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<212> TYPE: DNA

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 12

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tctgatgtag aaaaggatta gagatgctaa gagatagtga tgatatttca taaataatgt      180
aattctatat atgttaatta ccttttttgc gaggcattat tatgggtgaag gataagtttt      240
gaccatcaaa gaaggttaat gtggctgtgg tttcagggtc cataaagcct ttcaattcat      300
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<210> SEQ ID NO 13

<211> LENGTH: 4701

<212> TYPE: DNA

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 13

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtccct     180
tattagcctt gatagtgtcg aaaaaaagaa aaaaaacaaa aaaaagaat aaaaatacgg     240
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tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgcagtt gtctctatca	540
atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatcacgagat	600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcaggaatc	660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa	720
atataaagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa	780
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<210> SEQ ID NO 14

<211> LENGTH: 3126

<212> TYPE: DNA

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 14

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<210> SEQ ID NO 15

<211> LENGTH: 591

<212> TYPE: PRT

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 15

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Gly Ser Thr Val Pro Lys Ser Lys Ser Phe Glu Gln Asp Ser Arg Lys
35        40        45
Arg Thr Gln Ser Trp Thr Ala Leu Arg Val Gly Ala Ile Leu Ala Ala
50        55        60
Thr Ser Ser Val Ala Tyr Leu Asn Trp His Asn Gly Gln Ile Asp Asn
65        70        75        80
Glu Pro Lys Leu Asp Met Asn Lys Gln Lys Ile Ser Pro Ala Glu Val
85        90        95
Ala Lys His Asn Lys Pro Asp Asp Cys Trp Val Val Ile Asn Gly Tyr
100       105       110
Val Tyr Asp Leu Thr Arg Phe Leu Pro Asn His Pro Gly Gly Gln Asp
115      120      125
Val Ile Lys Phe Asn Ala Gly Lys Asp Val Thr Ala Ile Phe Glu Pro
130      135      140
Leu His Ala Pro Asn Val Ile Asp Lys Tyr Ile Ala Pro Glu Lys Lys
145      150      155      160
Leu Gly Pro Leu Gln Gly Ser Met Pro Pro Glu Leu Val Cys Pro Pro
165      170      175
Tyr Ala Pro Gly Glu Thr Lys Glu Asp Ile Ala Arg Lys Glu Gln Leu
180      185      190
Lys Ser Leu Leu Pro Pro Leu Asp Asn Ile Ile Asn Leu Tyr Asp Phe
195      200      205
Glu Tyr Leu Ala Ser Gln Thr Leu Thr Lys Gln Ala Trp Ala Tyr Tyr
210      215      220
Ser Ser Gly Ala Asn Asp Glu Val Thr His Arg Glu Asn His Asn Ala
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ctggtgacat tgcaagagc gacaaagatt ttgttatcgg ctttattgct caaagagaca 4620
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<210> SEQ ID NO 17

<211> LENGTH: 578

<212> TYPE: PRT

<213> ORGANISM: *Issatchenkia orientalis*

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<400> SEQUENCE: 17

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 Ala Lys Pro Thr Leu Lys Asn Asn Ser Arg Glu Ser Asn Lys Ser Arg
 35 40 45
 Asn Tyr Leu Ile Ala Ala Val Thr Ala Leu Ala Val Ser Thr Ser Ile
 50 55 60
 Gly Val Ala Val His Val Lys Asp Pro Leu Tyr Asn Asp Ala Thr Gly
 65 70 75 80
 Ser Asp Ser Pro Arg Ser Ile Ser Val Asp Glu Phe Val Lys His Asn
 85 90 95
 Ser Gln Asn Asp Cys Trp Ile Ala Ile Asn Gly Lys Val Tyr Asp Phe
 100 105 110
 Thr Asp Phe Ile Pro Asn His Pro Gly Gly Val Pro Pro Leu Val Asn
 115 120 125
 His Ala Gly Tyr Asp Gly Thr Lys Leu Tyr Glu Lys Leu His Pro Lys
 130 135 140
 Gly Thr Ile Glu Lys Phe Leu Pro Lys Asp Lys Phe Leu Gly Val Leu
 145 150 155 160
 Asp Gly Glu Ala Pro Lys Leu Glu Ala Asp Tyr Leu Val Asp Asp Asp
 165 170 175
 Glu Gln Glu Arg Leu Asp Tyr Leu Asn Asn Leu Pro Pro Leu Ser Ser
 180 185 190
 Ile Gln Asn Val Tyr Asp Phe Glu Tyr Leu Ala Lys Lys Ile Leu Pro
 195 200 205
 Lys Asp Ala Trp Ala Tyr Tyr Ser Cys Gly Ala Asp Asp Glu Ile Thr
 210 215 220
 Met Arg Glu Asn His Tyr Ala Tyr Gln Arg Val Tyr Phe Arg Pro Arg
 225 230 235 240
 Ile Cys Val Asp Val Lys Glu Val Asp Thr Ser Tyr Glu Met Leu Gly
 245 250 255
 Thr Lys Thr Ser Val Pro Phe Tyr Val Ser Ala Thr Ala Leu Ala Lys
 260 265 270
 Leu Gly His Pro Asp Gly Glu Cys Ser Ile Ala Arg Gly Ala Gly Lys
 275 280 285
 Glu Gly Val Val Gln Met Ile Ser Thr Leu Ser Ser Met Ser Leu Asp
 290 295 300
 Glu Ile Ala Ala Ala Arg Ile Pro Gly Ala Thr Gln Trp Phe Gln Leu
 305 310 315 320
 Tyr Ile Asn Glu Asp Arg Asn Val Ala Lys Gly Leu Val Lys His Ala
 325 330 335
 Glu Asp Leu Gly Met Lys Ala Ile Phe Ile Thr Val Asp Ala Pro Ser
 340 345 350
 Leu Gly Asn Arg Glu Lys Asp Lys Arg Leu Lys Phe Val Asn Asp Thr
 355 360 365
 Asp Val Asp Leu Gly Asp Ser Ala Asp Arg Asn Ser Gly Ala Ser Lys
 370 375 380
 Ala Leu Ser Ser Phe Ile Asp Ala Ser Val Ser Trp Asn Asp Val Lys
 385 390 395 400
 Ala Val Lys Ser Trp Thr Lys Leu Pro Val Leu Val Lys Gly Val Gln

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Thr Val Glu Asp Val Ile Glu Ala Tyr Asp Ala Gly Cys Gln Gly Val					
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Val Leu Ser Asn His Gly Gly Arg Gln Leu Asp Thr Ala Pro Pro Pro			440		445
	435				
Ile Glu Leu Leu Ala Glu Thr Val Pro Thr Leu Lys Arg Leu Gly Lys			455		460
	450				
Leu Arg Pro Asp Phe Glu Ile Leu Ile Asp Gly Gly Val Lys Arg Gly			470		480
	465				
Thr Asp Ile Leu Lys Ala Val Ala Ile Gly Gly Gln Asp Val Arg Val			490		495
	485				
Ser Val Gly Met Gly Arg Pro Phe Leu Tyr Ala Asn Ser Cys Tyr Gly			505		510
	500				
Glu Ala Gly Val Arg Lys Leu Ile Gln Asn Leu Lys Asp Glu Leu Glu			520		525
	515				
Met Asp Met Arg Leu Leu Gly Val Thr Lys Met Asp Gln Leu Ser Ser			535		540
	530				
Lys His Val Asp Thr Lys Arg Leu Ile Gly Arg Asp Ala Ile Asn Tyr			550		560
	545				
Leu Tyr Asp Asn Val Tyr Ser Pro Ile Glu Thr Val Lys Phe Asn Asn			570		575
	565				
Glu Asp					

<210> SEQ ID NO 18
 <211> LENGTH: 4419
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Aspergillus niger.

<400> SEQUENCE: 18

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtccct    180
tattagcctt gatagtgtg aaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg    300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatatat ctttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg    480
tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgcagtt gtctctatca    540
atgaaaattt cgaggaagac gataaggtta agataagtag ataagagaat gatacgagat    600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcaggaatc    660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa    720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gccaatcgaa    780
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atagcactcg agatctccc agtttatcat tatcaatact gccatttcaa agaatacgta    900
aataattaat agtagtgatt ttocctaactt tatttagtca aaaaattggc cttttaattc    960
tgctgtaacc cgtacatgcc caaaataggg ggcgggttac acagaatata taacatcata   1020
    
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catcagttca taggtccatt ctcttagcgc aactacacag aacaggggca caaacaggca	1200
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<210> SEQ ID NO 19

<211> LENGTH: 497

<212> TYPE: PRT

<213> ORGANISM: *Aspergillus niger*

<400> SEQUENCE: 19

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          20          25          30
Glu Phe Leu Pro Glu His Pro Gly Gly Gln Lys Ile Ile Leu Lys Tyr
          35          40          45
Ala Gly Lys Asp Ala Thr Glu Glu Phe Asp Pro Ile His Pro Pro Asp
          50          55          60
Thr Leu Asp Lys Tyr Leu Asp Ser Ser Lys His Leu Gly Glu Val Asp
          65          70          75          80
Met Thr Thr Val Glu Gln Glu Glu Lys Thr Ala Asp Pro Glu Glu Glu
          85          90          95
Ala Arg Gln Glu Arg Ile Lys Cys Met Pro Ser Leu Ala Ala Cys Tyr
          100          105          110
Asn Leu Met Asp Phe Glu Thr Val Ala Arg Ser Val Met Lys Lys Thr
          115          120          125
Ala Trp Ala Tyr Tyr Ser Ser Gly Ala Asp Asp Glu Ile Thr Met Arg
          130          135          140
Glu Asn His Ser Ala Phe His Lys Ile Trp Phe Arg Pro Arg Val Leu
          145          150          155          160
Val Asp Val Glu His Val Asp Phe Ser Thr Thr Met Leu Gly Thr Lys
          165          170          175
Val Ser Val Pro Phe Tyr Val Thr Ala Thr Ala Leu Gly Lys Leu Gly
          180          185          190

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Asn Pro Glu Gly Glu Val Val Leu Thr Arg Ala Ala His Thr His Asp
 195 200 205

Val Ile Gln Met Ile Pro Thr Leu Ala Ser Cys Ser Phe Asp Glu Ile
 210 215 220

Val Asp Ala Arg Gln Gly Asp Gln Val Gln Trp Leu Gln Leu Tyr Val
 225 230 235 240

Asn Lys Asp Arg Asn Ile Thr Lys Arg Ile Val Gln His Ala Glu Ala
 245 250 255

Arg Gly Cys Lys Gly Leu Phe Ile Thr Val Asp Ala Pro Gln Leu Gly
 260 265 270

Arg Arg Glu Lys Asp Met Arg Ser Lys Phe Ser Asp Val Gly Ser Asn
 275 280 285

Val Gln Ala Ser Gly Gly Ser Ser Val Asp Arg Ser Gln Gly Ala Ala
 290 295 300

Arg Ala Ile Ser Ser Phe Ile Asp Pro Ala Leu Ser Trp Lys Asp Ile
 305 310 315 320

Pro Trp Phe Gln Ser Ile Thr Lys Met Pro Ile Leu Leu Lys Gly Val
 325 330 335

Gln Cys Val Glu Asp Val Leu Arg Ala Val Glu Met Gly Val Gln Gly
 340 345 350

Val Val Leu Ser Asn His Gly Gly Arg Gln Leu Glu Phe Ala Arg Ser
 355 360 365

Ala Ile Glu Val Leu Ala Glu Val Met Pro Ile Leu Arg Glu Arg Gly
 370 375 380

Trp Glu Asn Lys Ile Glu Ile Tyr Ile Asp Gly Gly Ile Arg Arg Ala
 385 390 395 400

Thr Asp Met Leu Lys Ala Leu Cys Leu Gly Ala Lys Gly Val Gly Ile
 405 410 415

Gly Arg Pro Phe Leu Tyr Ala Met Ser Ala Tyr Gly Gln Pro Gly Val
 420 425 430

Glu Arg Ala Met Gln Leu Leu Lys Asp Glu Met Glu Met Asn Met Arg
 435 440 445

Leu Ile Gly Ala Thr Lys Ile Glu Glu Leu Asn Pro Ser Leu Ile Asp
 450 455 460

Val Arg Gly Leu Val Gly Gly His Ser Ala Pro Val Pro Ser Asp Thr
 465 470 475 480

Leu Thr Thr Gly Ala Tyr Asp Pro Leu Gln Ala Pro Arg Phe Ser Glu
 485 490 495

Lys

<210> SEQ ID NO 20
 <211> LENGTH: 4398
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Yarrowia lipolytica.

<400> SEQUENCE: 20

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attaattaga acaagaactt atttagaaca cgttcactact gagtaagaac tcttgtccct      180
tattagcctt gatagtgctg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg      240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg      300
    
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attggttgat tatgacac 4398
    
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<210> SEQ ID NO 21
<211> LENGTH: 490
<212> TYPE: PRT
<213> ORGANISM: Yarrowia lipolytica
    
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<400> SEQUENCE: 21

```

Met Val Ser Val Glu Glu Val Ala Lys His Asn Ser Lys Asp Ser Cys
1           5           10          15
Trp Val Ile Leu His Gly Lys Ala Tyr Asp Leu Thr Glu Phe Leu Pro
                20          25          30
Glu His Pro Gly Gly Gln Ala Ile Ile Leu Lys Tyr Ala Gly Lys Asp
                35          40          45
Ala Thr Lys Ala Phe Asp Pro Ile His Pro Arg Asp Val Val Asp Lys
50          55          60
    
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Phe Leu Asp Lys Asp Lys His Leu Gly Glu Val Thr Gly Glu Ile Ala
 65 70 75 80
 Ala Glu Glu Glu Glu Glu Val Thr Pro Glu Glu Lys Ala Arg Leu Glu
 85 90 95
 Arg Tyr Glu Asn Arg Pro Pro Leu Ser Gln Ile Phe Asn Ser Phe Asp
 100 105 110
 Phe Glu Tyr Val Ala Arg His Thr Met Ser Pro Asn Ala Trp Ala Tyr
 115 120 125
 Tyr Ser Ser Gly Ser Asp Asp Glu Ile Thr Val Arg Glu Asn His Arg
 130 135 140
 Ala Phe His Lys Ile Trp Phe Arg Pro Arg Val Leu Val Asp Val Lys
 145 150 155 160
 Asn Val Asp Ile Ser Thr Thr Met Leu Gly Thr Lys Ser Ser Val Pro
 165 170 175
 Phe Tyr Ile Thr Ala Thr Ala Leu Gly Lys Leu Gly His Pro Glu Gly
 180 185 190
 Glu Val Val Leu Thr Arg Gly Ala Asp Lys Met Asp Val Ile Gln Met
 195 200 205
 Ile Pro Thr Leu Ala Ser Cys Ser Phe Asp Glu Ile Val Asp Ala Ala
 210 215 220
 Thr Asp Lys Gln Thr Gln Trp Met Gln Leu Tyr Val Asn Met Asp Arg
 225 230 235 240
 Glu Val Thr Lys Lys Ile Val Gln His Ala Glu Lys Arg Gly Val Lys
 245 250 255
 Gly Leu Phe Ile Thr Val Asp Ala Pro Gln Leu Gly Arg Arg Glu Lys
 260 265 270
 Asp Met Arg Thr Lys Phe Gly Asp Pro Gly Ala Gln Val Gln Gln Ser
 275 280 285
 Asp Asp Ser Val Asp Arg Ser Gln Gly Ala Ala Arg Ala Ile Ser Ser
 290 295 300
 Phe Ile Asp Pro Ser Leu Ser Trp Lys Asp Ile Pro Trp Phe Gln Ser
 305 310 315 320
 Ile Thr Lys Met Pro Ile Ile Leu Lys Gly Val Gln Cys Ala Glu Asp
 325 330 335
 Ala Leu Lys Ala Val Glu Tyr Lys Val Asp Gly Ile Leu Leu Ser Asn
 340 345 350
 His Gly Gly Arg Gln Leu Glu Phe Ala Arg Pro Ser Ile Glu Val Leu
 355 360 365
 Val Glu Val Met Ala Ala Leu Arg Ala Lys Gly Trp Gln Asp Tyr Ile
 370 375 380
 Glu Val Tyr Ile Asp Gly Gly Ile Arg Arg Ala Thr Asp Val Ile Lys
 385 390 395 400
 Ala Leu Cys Leu Gly Ala Lys Gly Val Gly Ile Gly Arg Pro Phe Leu
 405 410 415
 Tyr Ala Met Ser Thr Tyr Gly Glu Asp Gly Val Cys His Leu Ile Gln
 420 425 430
 Leu Leu Lys Asp Glu Met Glu Met Asn Met Arg Leu Ile Gly Ala Thr
 435 440 445
 Lys Ile Glu Asp Leu Asn Pro Ser Met Val Asp Leu Lys Ser Ile Phe
 450 455 460
 Thr His Ser Ala Asp Thr Ala Arg Asp Ile Leu Gly Glu Asn Val Tyr
 465 470 475 480

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Gln His Met Glu Met Pro Leu Phe Lys Gly
485 490

<210> SEQ ID NO 22

<211> LENGTH: 2919

<212> TYPE: DNA

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 22

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtccct 180
tattagcctt gatagtctg aaaaaagaa aaaaaacaaa aaaaagaat aaaataacg 240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg 300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag 360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc 420
aataatata cttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg 480
tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgcagtt gtctctatca 540
atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat 600
aaagcacaaa tttagcagaaa gaagagtggg tgccgaacaga gtaaaccgaa tcagggaatc 660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa 720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa 780
agtttatttc agagttcttc agacttctta actcctgtaa aaacaaaaaa aaaaaaggc 840
atagcactcg agatctccc agtttatcat tatcaatact gccatttcaa agaatacgt 900
aataattaat agtagtgatt ttccctaact tatttagtca aaaaattggc cttttaattc 960
tgctgtaacc cgtacatgcc caaaataggg ggccgggttac acagaatata taacatcata 1020
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catcagttca taggtccatt ctcttagcgc aactacacag aacaggggca caaacaggca 1200
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gcaattgacc tacgcatgta tctatctcat tttcttacac cttctattac cttctgctct 1320
ctctgatttg gaaaaagctg aaaaaaagg ttgaaaccag ttcctgaaa ttattcccct 1380
atgtgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc 1440
ttaaacttct taaattctac ttttatagtt agtctttttt ttagttttaa acaccaagaa 1500
cttagtttgc aataaacaca cataaacaaa caaatctagt aaacaggccc cttttccttt 1560
gtcgatatca tgtaattagt tatgtcacgc ttacattcac gccctcctcc cacatccgct 1620
ctaaccgaaa aggaaggagt tagacaacct gaagtctagg tccctattta tttttttata 1680
gttatgtagg tattaagaac gttatttata tttcaaattt ttcttttttt tctgtacaaa 1740
cgctgtacg catgtaacgg gcagacggcc ggccataact tcgtataatg tatgctatac 1800
gaagttatgg caacggttca tcatctcatg gatctgcaca tgaacaaa caagagtcaa 1860
acgacgttga aattgaggct actgcgcaa ttgatgacaa tacagacgat gataacaaac 1920
cgaagtatc tgatgtagaa aaggattaga gatgctaaga gatagtgatg atatttcata 1980
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acagaacaaa aacctgcagg aaacgaagat aatcatgtc gaaagctaca tataaggaac	2340
gtgctgctac tcacctagt cctgttgctg ccaagctatt taatatcatg cacgaaaagc	2400
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aagcattagg tcccaaaatt tgtttactaa aaacacatgt ggatatcttg actgattttt	2520
ccatggaggg cacagttaag ccgctaaagg cattatccgc caagtacaat tttttactct	2580
tcgaagacag aaaatttgc gacattggtg atacagtcaa attgcagtac tctgcgggtg	2640
tatacagaat agcagaatgg gcagacatta cgaatgcaca cgggtgtggtg ggcccaggta	2700
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tgtagcaga attgtcatgc aagggtccc tagctactgg agaataact aagggtactg	2820
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<210> SEQ ID NO 23

<211> LENGTH: 4700

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Issatchenkia orientalis.

<400> SEQUENCE: 23

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gtatacagaa tagcagaatg gccagacatt acgaatgcac acggtgtggt gggcccagggt	180
attgttagcg gtttgaagca gcggcggaag gaagtaacaa aggaacctag aggccttttg	240
atgttagcag aattgtcatg caagggctcc ctactactg gagaatatac taagggtact	300
gttgacattg cgaagagcga caaagatttt gttatcggct ttattgctca aagagacatg	360
ggtggaagag atgaaggtta cgattggtg attatgacac ccggtgtggg tttagatgac	420
aagggagacg cattgggtca acagtataga accgtggatg atgtggtctc tacaggatct	480
gacattatta ttgttgaag aggactattt gcaaaaggaa gggatgctaa ggtagagggt	540
gaacgttaca gaaaagcagg ctgggaagca tatttgagaa gatgcccga gcaaaactaa	600
aaaactgtat tataagtaaa tgcattgtata ctaaactcac aaattagagc ttcaatttaa	660
ttatatcagt tattaccogg gaatctcggc cgtaagtatt tttataatga cgaaaaaaaa	720
aaaattgaa agaaaaagct tcattgacct tataaaaagg aacctccaa tacctcgcca	780
gaaccaagta acagtatttt acggggcaca aatcaagaac aataagacag gactgtaaag	840
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atgaaggatt tcattcgttt gataacttcg tataatgtat gctatacga gttatctcga	960
gatctcccct aaaccgtgga atatttcgga tacccttttg ttgtttccgg gtgtacaata	1020
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cagcgaattt cctcacatgt agggaccgaa ttgtttacaa gttctctgta ccaccatgga	3480
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<210> SEQ ID NO 24

<211> LENGTH: 523

<212> TYPE: PRT

<213> ORGANISM: *Issatchenkia orientalis*

<400> SEQUENCE: 24

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Met Ser Asp Leu Glu Ser Gln His Thr Arg Glu Glu Leu Asp Lys Ser
 1          5          10          15
Glu Asp Glu Glu Ile Gln Leu Ile Asn Glu Ser Arg Pro Pro Lys Leu
 20          25          30
Asp Lys Glu Thr Ile Lys Arg Tyr Leu Lys Thr Arg Phe Thr Glu Leu
 35          40          45
Leu Pro Thr Lys Gln Gln Met Lys Gln Asn Lys His Leu Leu Asn Pro
 50          55          60
Leu Pro Gly Leu Arg Met Met Gly Arg Arg Gln Trp Leu Met Phe Leu
 65          70          75          80
Ser Ala Phe Cys Ala Trp Thr Trp Asp Ala Tyr Asp Phe Phe Thr Val
 85          90          95
Ser Leu Asn Thr Val Gln Leu Ala Lys Asp Phe Asp Lys Asn Val Lys
 100         105         110
Asp Ile Thr Trp Gly Ile Thr Leu Val Leu Met Leu Arg Ser Val Gly
 115         120         125
Gly Ile Thr Phe Gly Tyr Leu Gly Asp Arg Tyr Gly Arg Lys Trp Pro
 130         135         140
Leu Val Ala Asn Leu Leu Ile Val Cys Ile Leu Glu Ile Gly Ser Gly
 145         150         155         160

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Phe Ile Lys Thr Tyr Arg Gln Phe Leu Gly Val Arg Ala Leu Phe Gly
 165 170 175

Ile Cys Leu Gly Gly Val Tyr Gly Asn Ala Thr Ala Thr Ala Leu Asp
 180 185 190

Asp Cys Pro Thr Glu Ala Arg Gly Phe Ile Gly Gly Leu Leu Gln Gln
 195 200 205

Gly Tyr Ala Phe Gly Tyr Leu Leu Ala Val Val Phe Lys Arg Ala Ile
 210 215 220

Ala Asp Asn Ser Ser His Lys Trp Arg Ala Thr Phe Trp Phe Gly Ala
 225 230 235 240

Gly Ile Ser Phe Leu Phe Ala Leu Phe Arg Ala Ser Leu Pro Glu Thr
 245 250 255

Lys Ala Phe Leu Arg Lys Lys Glu Ile Glu Arg Tyr Asn Lys Glu His
 260 265 270

Gly Ile Tyr Gln Pro Thr Phe Gln Met Lys Ile Val Glu Ser Leu Lys
 275 280 285

Asn Tyr Trp Leu Met Met Ile Tyr Leu Val Leu Phe Met Ser Gly Phe
 290 295 300

Ser Phe Met Ser His Ser Ser Gln Asp Leu Tyr Pro Thr Leu Leu Thr
 305 310 315 320

Val Arg Tyr Ser Phe Ser Glu Asn Lys Ala Thr Val Thr Asn Cys Val
 325 330 335

Ala Asn Ile Gly Ala Phe Ile Gly Gly Val Phe Thr Gly His Ile Ser
 340 345 350

Asn Phe Leu Gly Arg Arg Leu Thr Ile Met Leu Ile Cys Ile Phe Gly
 355 360 365

Gly Ala Leu Ile Tyr Pro Trp Ala Phe Ile Asp Gly Thr Gly Ile Asn
 370 375 380

Ala Gly Val Phe Phe Leu Gln Trp Cys Val Gln Gly Gly Leu Gly Val
 385 390 395 400

Val Pro Ser His Leu Ser Glu Leu Ala Pro Pro Asp Leu Arg Ala Phe
 405 410 415

Val Val Gly Ile Ser Tyr Gln Leu Gly Asn Leu Ala Ala Ser Ala Ser
 420 425 430

Ser Thr Ile Glu Thr Thr Ile Gly Glu Gln Phe Pro Met Thr Ser Pro
 435 440 445

Ser Gly Glu Pro Ile Tyr Asp Tyr Ala Lys Val Met Ala Ile Phe Val
 450 455 460

Gly Cys Val Phe Ala Phe Val Leu Ile Val Thr Leu Phe Gly Pro Glu
 465 470 475 480

Arg Arg Asn Ala Ser Phe Glu Asn Ala Val Thr Thr Asn Glu Glu Phe
 485 490 495

Gly Asn Ser Ala Gly Ala Val Asp Lys Ile Glu His Val Glu Glu Cys
 500 505 510

Ile Val Pro Lys His Ala Arg Asn Gln Gln Cys
 515 520

<210> SEQ ID NO 25
 <211> LENGTH: 4979
 <212> TYPE: DNA
 <213> ORGANISM: Saccharomyces cerevisiae
 <400> SEQUENCE: 25

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attgttagcg gtttgaagca ggcggcggaa gaagtaacaa aggaacctag aggccttttg	240
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gttgacattg cgaagagcga caaagatttt gttatcggtt ttattgctca aagagacatg	360
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<210> SEQ ID NO 26
<211> LENGTH: 616
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae
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<400> SEQUENCE: 26
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Pro Leu Val Asp Gly Glu Gly Asn Pro Ile Asn Tyr Glu Pro Glu Val
35          40          45
Tyr Asn Pro Asp His Glu Lys Leu Tyr His Asn Pro Ser Leu Pro Ala
50          55          60
Gln Ser Ile Gln Asp Thr Arg Asp Asp Glu Leu Leu Glu Arg Val Tyr
65          70          75          80
Ser Gln Asp Gln Gly Val Glu Tyr Glu Glu Asp Glu Glu Asp Lys Pro
85          90          95
Asn Leu Ser Ala Ala Ser Ile Lys Ser Tyr Ala Leu Thr Arg Phe Thr
100         105        110
Ser Leu Leu His Ile His Glu Phe Ser Trp Glu Asn Val Asn Pro Ile
115        120        125
Pro Glu Leu Arg Lys Met Thr Trp Gln Asn Trp Asn Tyr Phe Phe Met
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Gly Tyr Phe Ala Trp Leu Ser Ala Ala Trp Ala Phe Phe Cys Val Ser
145        150        155        160
Val Ser Val Ala Pro Leu Ala Glu Leu Tyr Asp Arg Pro Thr Lys Asp
165        170        175
Ile Thr Trp Gly Leu Gly Leu Val Leu Phe Val Arg Ser Ala Gly Ala
180        185        190
Val Ile Phe Gly Leu Trp Thr Asp Lys Ser Ser Arg Lys Trp Pro Tyr
195        200        205
Ile Thr Cys Leu Phe Leu Phe Val Ile Ala Gln Leu Cys Thr Pro Trp
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Cys Asp Thr Tyr Glu Lys Phe Leu Gly Val Arg Trp Ile Thr Gly Ile
225        230        235        240
Ala Met Gly Gly Ile Tyr Gly Cys Ala Ser Ala Thr Ala Ile Glu Asp
245        250        255
Ala Pro Val Lys Ala Arg Ser Phe Leu Ser Gly Leu Phe Phe Ser Ala
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Tyr Ala Met Gly Phe Ile Phe Ala Ile Ile Phe Tyr Arg Ala Phe Gly
275        280        285
Tyr Phe Arg Asp Asp Gly Trp Lys Ile Leu Phe Trp Phe Ser Ile Phe
290        295        300
Leu Pro Ile Leu Leu Ile Phe Trp Arg Leu Leu Trp Pro Glu Thr Lys
305        310        315        320
Tyr Phe Thr Lys Val Leu Lys Ala Arg Lys Leu Ile Leu Ser Asp Ala
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<210> SEQ ID NO 28

<211> LENGTH: 600

<212> TYPE: PRT

<213> ORGANISM: Kluyveromyces lactis

<400> SEQUENCE: 28

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 Gly Ser Val Leu Ser Arg Val Ala Ser Tyr Val Ser Arg Lys Asp Arg
 50 55 60
 Tyr Val Asp Glu Asn Gly Asn Glu Val Trp Gln Asp Asp Glu Val Ser
 65 70 75 80
 Ile Leu Met Glu Glu Asp Glu Thr Pro Asp Phe Thr Trp Lys Asn Ile
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 Arg His Tyr Ala Ile Thr Arg Phe Thr Thr Leu Thr Glu Leu His Arg
 100 105 110
 Val Ser Met Glu Asn Ile Asn Pro Ile Pro Glu Leu Arg Lys Met Thr
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 210 215 220
 Leu Gly Val Arg Trp Ile Ser Gly Ile Ala Met Gly Gly Ile Tyr Ala
 225 230 235 240
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 Ala Ile Ile Phe Tyr Arg Ala Phe Leu Asn Val Asn Gly Glu Asn Tyr
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 Lys Ala Arg Gln Leu Met Arg Asp Asp Ala Ile Ala Lys Asn Gly Gly
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 370 375 380
 Thr Met Leu Arg Ala Gln Leu Arg Phe Ser Glu Asp Ala Val Thr Val
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 Gly Gln Leu Met Glu Ile Thr Gly Arg Arg Val Gly Leu Leu Leu Ala

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Pro	Ile	Glu	Trp	Asn	Ser	Ala	Gly	Glu	Val	Thr	Asn	Lys	Asp	Tyr	Ser
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Lys	Val	Met	Ala	Ile	Leu	Thr	Gly	Ser	Ser	Val	Ile	Phe	Thr	Phe	Val
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 <211> LENGTH: 3981
 <212> TYPE: DNA
 <213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 29

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<210> SEQ ID NO 30
<211> LENGTH: 293
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae
    
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<400> SEQUENCE: 30

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Glu Asp Glu Arg Pro Ser His Asp Ser Leu Gly Lys Ile Tyr Thr Gly
35         40         45
Gly Asp Asn Asn Glu Tyr Ile Tyr Ile Gly Arg Gln Lys Phe Leu Lys
50         55         60
Ser Asp Leu Tyr Gln Ala Phe Gly Gly Thr Leu Asn Pro Gly Leu Ala
65         70         75         80
Pro Ala Pro Val His Lys Phe Ala Asn Pro Ala Pro Leu Gly Leu Ser
85         90         95
Ala Phe Ala Leu Thr Thr Phe Val Leu Ser Met Phe Asn Ala Arg Ala
100        105        110
Gln Gly Ile Thr Val Pro Asn Val Val Val Gly Cys Ala Met Phe Tyr
115        120        125
Gly Gly Leu Val Gln Leu Ile Ala Gly Ile Trp Glu Ile Ala Leu Glu
130        135        140
Asn Thr Phe Gly Gly Thr Ala Leu Cys Ser Tyr Gly Gly Phe Trp Leu
145        150        155        160
Ser Phe Ala Ala Ile Tyr Ile Pro Trp Phe Gly Ile Leu Glu Ala Tyr
165        170        175
Glu Asp Asn Glu Ser Asp Leu Asn Asn Ala Leu Gly Phe Tyr Leu Leu
180        185        190
Gly Trp Ala Ile Phe Thr Phe Gly Leu Thr Val Cys Thr Met Lys Ser
195        200        205
Thr Val Met Phe Phe Leu Leu Phe Phe Leu Val Ala Leu Thr Phe Leu
210        215        220
Leu Leu Ser Ile Gly His Phe Ala Asn Arg Leu Gly Val Thr Arg Ala
225        230        235        240
Gly Gly Val Leu Gly Val Val Val Ala Phe Ile Gly Trp Tyr Asn Ala
245        250        255
Tyr Ala Gly Val Ala Thr Lys Gln Asn Ser Tyr Val Leu Ala Arg Pro
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Phe Pro Leu Pro Ser Thr Glu Arg Val Ile Phe Leu Ile Asn Leu Pro
    
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<210> SEQ ID NO 31			
<211> LENGTH: 3921			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Sequence put together from multiple organisms: Saccharomyces cerevisiae and Aggregatibacter actinomycetemcomitans.			
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<210> SEQ ID NO 32

<211> LENGTH: 331

<212> TYPE: PRT

<213> ORGANISM: Aggregatibacter actinomycetemcomitans

<400> SEQUENCE: 32

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Met Leu Ser	Glu Arg Thr Ala Lys	Met Ala Glu His	Cys Glu Ala Val
	35	40	45
Cys Ile Phe	Val Asn Asp Asp	Gly Ser Arg Lys	Val Leu Glu Lys Leu
	50	55	60
Ala Ala Leu	Gly Val Lys Ile Ile	Ala Leu Arg Cys	Ala Gly Phe Asn
	65	70	75
Asn Val Asp	Leu Lys Ala Ala	Gln Glu Leu Gly	Leu Thr Val Val Arg
	85	90	95
Val Pro Ala	Tyr Ser Pro Glu	Ala Val Ala Glu	His Thr Val Gly Leu
	100	105	110
Met Met Thr	Leu Asn Arg Arg	Ile His Arg Ala	Tyr Gln Arg Thr Arg
	115	120	125
Glu Ala Asn	Phe Ser Leu Glu	Gly Leu Ile Gly	Phe Asn Met Tyr Gly
	130	135	140
Arg Thr Val	Gly Val Ile Gly	Thr Gly Lys Ile	Gly Val Ala Val Met
	145	150	155
Arg Ile Leu	Lys Gly Phe Gly	Met His Ile Leu	Ala Phe Asp Pro Phe
	165	170	175
Lys Asn Pro	Thr Ala Glu Glu	Leu Gly Ala Glu	Tyr Val Ser Leu Asp
	180	185	190
Glu Ile Tyr	Arg Arg Ser His	Val Ile Thr Leu	His Cys Pro Ala Thr
	195	200	205
Pro Glu Asn	Tyr His Leu Leu	Asn Arg Glu Ala	Phe Ala Lys Met Lys
	210	215	220
Asp Gly Val	Met Ile Ile Asn	Thr Ser Arg Gly	Thr Leu Ile Asp Thr
	225	230	235
Lys Ala Ala	Ile Asp Ala Leu	Lys Gln Arg Lys	Ile Gly Ala Leu Gly
	245	250	255
Met Asp Val	Tyr Glu Asn Glu	Arg Asp Leu Phe	Phe Glu Asp Lys Ser
	260	265	270
Asn Glu Val	Ile Leu Asp Asp	Val Phe Arg Arg	Leu Ser Ser Cys His
	275	280	285
Asn Val Leu	Leu Thr Gly His	Gln Ala Phe Leu	Thr Glu Glu Ala Leu
	290	295	300
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<210> SEQ ID NO 33
 <211> LENGTH: 3924
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Ornithorhynchus anatinus.

<400> SEQUENCE: 33

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gtgttgaaga aacatgaat tgcccagtat tcttaacca actgcacaga aaaaaaacct 3300
gcagaaaacg aagataaatc atgtcgaaag ctacatataa ggaacgtgct gctactcatc 3360
ctagtcctgt tgctgccaag ctatttaata tcatgcacga aaagcaaca aacttgtgtg 3420
cttcattgga tgttcgtacc accaaggaat tactggagtt agttgaagca ttaggtccca 3480
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aatgggcaga cattacgaat gcacacggtg tgggtggccc aggtattgtt agcggtttga 3720
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catgcaaggg ctccttagct actggagaat atactaaggg tactgttgac attgcgaaga 3840
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gttacgattg gttgattatg acac 3924

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<210> SEQ ID NO 34
<211> LENGTH: 332
<212> TYPE: PRT
<213> ORGANISM: Ornithorhynchus anatinus

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<400> SEQUENCE: 34
Met Ala Gly Val Lys Glu Gln Leu Ile Gln Asn Leu Leu Lys Glu Glu
1          5          10         15
Tyr Ala Pro Gln Asn Lys Ile Thr Val Val Gly Val Gly Ala Val Gly
20         25         30
Met Ala Cys Ala Ile Ser Ile Leu Met Lys Asp Leu Ala Asp Glu Leu
35         40         45
Ala Leu Val Asp Val Ile Glu Asp Lys Leu Lys Gly Glu Met Met Asp
50         55         60
Leu Gln His Gly Ser Leu Phe Leu Arg Thr Pro Lys Ile Val Ser Gly
65         70         75         80
Lys Asp Tyr Ser Val Thr Ala Asn Ser Lys Leu Val Ile Ile Thr Ala
85         90         95
Gly Ala Arg Gln Gln Glu Gly Glu Ser Arg Leu Asn Leu Val Gln Arg
100        105        110
Asn Val Asn Ile Phe Lys Phe Ile Ile Pro Asn Val Val Lys Tyr Ser
115        120        125

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Pro Asn Cys Lys Leu Leu Val Val Ser Asn Pro Val Asp Ile Leu Thr
 130 135 140

Tyr Val Ala Trp Lys Ile Ser Gly Phe Pro Lys Asn Arg Val Ile Gly
 145 150 155 160

Ser Gly Cys Asn Leu Asp Ser Ala Arg Phe Arg Tyr Leu Met Gly Glu
 165 170 175

Arg Leu Gly Ile His Ser Thr Ser Cys His Gly Trp Val Ile Gly Glu
 180 185 190

His Gly Asp Ser Ser Val Pro Val Trp Ser Gly Val Asn Val Ala Gly
 195 200 205

Val Ser Leu Lys Asn Leu His Pro Asp Leu Gly Thr Asp Ala Asp Lys
 210 215 220

Glu Gln Trp Lys Asp Val His Lys Gln Val Val Asp Ser Ala Tyr Glu
 225 230 235 240

Val Ile Lys Leu Lys Gly Tyr Thr Ser Trp Ala Ile Gly Leu Ser Val
 245 250 255

Ala Asp Leu Ala Glu Ser Ile Val Lys Asn Leu Arg Arg Val His Pro
 260 265 270

Ile Ser Thr Met Ile Lys Gly Leu Tyr Gly Ile Lys Asp Glu Val Phe
 275 280 285

Leu Ser Val Pro Cys Val Leu Gly Gln Asn Gly Ile Ser Asp Val Val
 290 295 300

Lys Ile Thr Leu Lys Ser Glu Glu Glu Ala His Leu Lys Lys Ser Ala
 305 310 315 320

Asp Thr Leu Trp Gly Ile Gln Lys Glu Leu Gln Phe
 325 330

<210> SEQ ID NO 35

<211> LENGTH: 4641

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Acetobacter acetii.

<400> SEQUENCE: 35

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtcctt   180
tattagcctt gatagtgctg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg   240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg   300
gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag   360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc   420
aataatata cttttagtat aaccctgaaa tttgcctat agaaatctag ggtttctgtg   480
tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgcagtt gtctctatca   540
atgaaaaatt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat   600
aaagcacaaa tttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcagggaatc   660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa   720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa   780
agtttatttc agagttcttc agacttctta actcctgtaa aaacaaaaaa aaaaaaaggc   840
atagcactcg agatctcccg agtttatcat tatcaatact gccatttcaa agaatacgt   900

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aataattaat	agtagtgatt	ttcctaactt	tatttagtca	aaaaattggc	cttttaattc	960
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ggtgtctggg	tgaacagttt	attcctggca	tccactaaat	ataatggagc	ccgctttttt	1080
taagctggca	tccagaaaaa	aaaagaatcc	cagcaccaaa	atattgtttt	cttcaccaac	1140
catcagttca	taggtccatt	ctcttagcgc	aactacacag	aacaggggca	caaacaggca	1200
aaaaacgggc	acaacctcaa	tggagtgatg	caacctgctt	ggagtaaata	atgacacaag	1260
gcaattgacc	tacgcatgta	tctatctcat	tttcttacac	cttctattac	cttctgctct	1320
ctctgatttg	gaaaaagctg	aaaaaaaaag	tgaaaccag	ttccctgaaa	ttattcccct	1380
atgtgactaa	taagtatata	aagacggtag	gtattgattg	taattctgta	aatctatttc	1440
ttaaacttct	taaattctac	ttttatagtt	agtctttttt	ttagtttaaa	acaccaagaa	1500
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caacaagcaa	ttacttgaac	aactgaaaaa	catagctggt	caacatcacg	ttctaacagg	1620
tgaagattct	acttacagat	tcacacacgg	ttttagattt	ggtgctggaa	aagttctagc	1680
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ggcaagtgtc	ttgggaggcg	tgtctaacaa	ctctgggggt	gccttgggtc	aaagagggcc	2040
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cgccgttgaa	cacgagatgt	tgccaggttt	ggatgccaga	ggtgctagat	accagccga	3120
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aggcggcgga agaagtaaca aaggaaccta gaggcctttt gatgtagca gaattgtcat 4500
gcaagggctc cctagctact ggagaatata ctaagggtag tgttgacatt gcgaagagcg 4560
acaaagattt tgttatcggc tttattgctc aaagagacat gggtggaaga gatgaaggtt 4620
acgattgggt gattatgaca c 4641

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<210> SEQ ID NO 36

<211> LENGTH: 571

<212> TYPE: PRT

<213> ORGANISM: Acetobacter aceti

<400> SEQUENCE: 36

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Met Thr Thr Ala Ala Glu Asn Asn Lys Gln Leu Leu Glu Gln Leu Lys
1          5          10          15
Asn Ile Ala Gly Gln His His Val Leu Thr Gly Glu Asp Ser Thr Tyr
20        25        30
Arg Phe Thr His Gly Phe Arg Phe Gly Ala Gly Lys Val Leu Ala Val
35        40        45
Val Gln Pro Gly Ser Leu Ile Glu Leu Trp Arg Val Ala Asp Ala Cys
50        55        60
Val Asn Ala Asp Lys Ile Ile Ile Met Gln Ala Ala Asn Thr Gly Leu
65        70        75        80
Thr Gly Gly Ser Thr Pro Asp Gly Asn Asn Tyr Asp Arg Asp Ile Val
85        90        95
Leu Ile Ser Thr Leu Arg Leu Asp Lys Ser His Leu Ile Gly Asn Gly
100       105       110
Lys Gln Val Val Cys Leu Pro Gly Ala Thr Leu Tyr Glu Leu Glu Ser
115       120       125

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Lys Leu Ala Ala Ile Gly Arg Glu Pro His Ser Val Ile Gly Ser Ser
 130 135 140
 Cys Ile Gly Ala Ser Val Leu Gly Gly Val Ser Asn Asn Ser Gly Gly
 145 150 155 160
 Ala Leu Val Gln Arg Gly Pro Ala Tyr Thr Glu Met Ala Val Phe Gly
 165 170 175
 Gln Val Gly Lys Asp Gly Lys Leu Gln Leu Val Asn His Leu Gly Ile
 180 185 190
 Lys Leu Gly Asn Asp Pro Glu Ala Ile Leu Gln Lys Leu Glu Ala Gly
 195 200 205
 Thr Phe Thr Glu Ala Asp Ile Asp Trp Asp Ala Gly Lys Gly His Asp
 210 215 220
 Gly Asp Tyr Ile Asn His Val Arg Glu Ile Asp Ala Asp Thr Pro Ala
 225 230 235 240
 Arg Tyr Asn Ala Asp Pro Ala Arg Trp Tyr Glu Ala Ala Gly Cys Ala
 245 250 255
 Gly Lys Leu Val Val Phe Ala Val Arg Leu Asp Thr Phe Pro Ala Asn
 260 265 270
 Lys Asp Thr Ser Val Phe Tyr Ile Gly Thr Asn Asn Thr Asp Glu Leu
 275 280 285
 Glu Asn Leu Arg Arg His Val Leu Thr Ala Phe Asp Glu Leu Pro Ile
 290 295 300
 Cys Gly Glu Tyr Ile His Arg Asp Ala Phe Asn Ile Ala Glu Lys Tyr
 305 310 315 320
 Gly Lys Asp Thr Phe Ala Val Ile Arg Tyr Phe Gly Thr Lys Phe Leu
 325 330 335
 Pro Lys Met Phe Ala Met Lys Ser Arg Phe Asp Ile Phe Ala Lys Lys
 340 345 350
 Leu Gly Phe Leu Pro Asp His Leu Ser Asp Arg Ile Met Gln Ala Leu
 355 360 365
 Ser Thr Leu Leu Pro Arg Gln Leu Pro Gln Arg Met Leu Asp Tyr Arg
 370 375 380
 Asp Lys Tyr Glu His His Leu Met Leu Lys Val Ser His Asn Leu Ala
 385 390 395 400
 Gly Pro Met Arg Gln Tyr Leu Gln Thr Tyr Phe Gly Ser Ala Thr Gly
 405 410 415
 Asp Tyr Phe Glu Cys Thr Pro Glu Glu Gly Lys Leu Ala Phe Leu His
 420 425 430
 Arg Phe Ala Ala Ala Gly Ala Ala Val Arg Tyr Arg Ala Val His His
 435 440 445
 Lys Glu Ala Glu Asp Ile Val Ala Leu Asp Val Ala Leu Arg Arg Asn
 450 455 460
 Asp Arg Glu Trp Phe Glu Lys Leu Pro Gln Asp Ile Glu Asn Lys Leu
 465 470 475 480
 Ile Val Lys Leu Tyr Tyr Gly His Phe Leu Cys His Val Met His Gln
 485 490 495
 Asp Tyr Val Val Lys Lys Gly Tyr Asn Ala Ile Ala Val Glu His Glu
 500 505 510
 Met Leu Pro Gly Leu Asp Ala Arg Gly Ala Arg Tyr Pro Ala Glu His
 515 520 525
 Asn Val Gly His Leu Tyr Lys Ala Pro Gln Asn Met Leu Asp His Tyr
 530 535 540

-continued

Gln Gln Leu Asp Pro Cys Asn Cys Met Asn Pro Gly Ile Gly Gln Gly
 545 550 555 560

Thr Lys Arg Lys Asn Trp Val Ala Glu Ser Val
 565 570

<210> SEQ ID NO 37
 <211> LENGTH: 4360
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Gluconobacter oxydans.

<400> SEQUENCE: 37

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gtatttccga gatcacgatta ctccagttcc tcttacaaga aatgcataaa aatagttaca    120
attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtcctt    180
tattagcctt gatagtgtctg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcaacttactg    300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatatat cttttagtat aacctgaaa tttgcoctat agaaatctag ggtttctgtg    480
tggtttccgg gtgagtcata cggtcttttt gaatttcttt ttttgcagtt gtctctatca    540
atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatcacgagat    600
aaagcacaaa ttacgagaaa gaagagtggg tgccaacaga gtaaaccgaa tcaggggaatc    660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa    720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa    780
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tgctgtaac ccgtacatgcc caaaataggg ggccgggttac acagaatata taacatcata    1020
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taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccaac    1140
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atgtgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc    1440
ttaaacttct taaattctac ttttatagtt agtctttttt ttagttttaa acaccaagaa    1500
cttagtttgg aataaacaca cataaacaaa caaatctaga atgccagaac cagttatgac    1560
agcctcctct gcactctgctc cagatagact acaagccgta ttgaaagcct tacagccagt    1620
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tatgaacgct tccaaccttc ctgaagcagt agtttttgcct gaatctactc aagatgttgc    1740
aaccgttctc agacactgcc acgaatggcg tgtcccagtc gttgcattcg gtgcaggaac    1800
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tggtgaggca	acaatcggtg	gaatgtgtgc	cacaagagct	tcaggtacag	cagcagttag	2040
atacgggtaca	atgaagaaa	atgttttagg	actcacagtg	gttttagcca	ctggtgaaat	2100
cattagaaca	ggaggaagag	tcagaaagtc	tagtacagga	tatgatttaa	cctccttggt	2160
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ccatggaggg	cacagttaag	ccgctaaagg	cattatccgc	caagtacaat	tttttactct	4020
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tgttagcaga	attgtcatgc	aagggctccc	tagctactgg	agaatatact	aagggtagctg	4260

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gtggaagaga tgaaggttac gattggttga ttatgacacg 4360

<210> SEQ ID NO 38

<211> LENGTH: 477

<212> TYPE: PRT

<213> ORGANISM: Gluconobacter oxydans

<400> SEQUENCE: 38

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 Ser Thr Ala Pro Ser Val Arg Glu Glu His Ser His Gly Glu Ala Met
 35 40 45
 Asn Ala Ser Asn Leu Pro Glu Ala Val Val Phe Ala Glu Ser Thr Gln
 50 55 60
 Asp Val Ala Thr Val Leu Arg His Cys His Glu Trp Arg Val Pro Val
 65 70 75 80
 Val Ala Phe Gly Ala Gly Thr Ser Val Glu Gly His Val Val Pro Pro
 85 90 95
 Glu Gln Ala Ile Ser Leu Asp Leu Ser Arg Met Thr Gly Ile Val Asp
 100 105 110
 Leu Asn Ala Glu Asp Leu Asp Cys Arg Val Gln Ala Gly Ile Thr Arg
 115 120 125
 Gln Thr Leu Asn Val Glu Ile Arg Asp Thr Gly Leu Phe Phe Pro Val
 130 135 140
 Asp Pro Gly Gly Glu Ala Thr Ile Gly Gly Met Cys Ala Thr Arg Ala
 145 150 155 160
 Ser Gly Thr Ala Ala Val Arg Tyr Gly Thr Met Lys Glu Asn Val Leu
 165 170 175
 Gly Leu Thr Val Val Leu Ala Thr Gly Glu Ile Ile Arg Thr Gly Gly
 180 185 190
 Arg Val Arg Lys Ser Ser Thr Gly Tyr Asp Leu Thr Ser Leu Phe Val
 195 200 205
 Gly Ser Glu Gly Thr Leu Gly Ile Ile Thr Glu Val Gln Leu Arg Leu
 210 215 220
 His Gly Arg Pro Asp Ser Val Ser Ala Ala Ile Cys Gln Phe Glu Ser
 225 230 235 240
 Leu His Asp Ala Ile Gln Thr Ala Met Glu Ile Ile Gln Cys Gly Ile
 245 250 255
 Pro Ile Thr Arg Val Glu Leu Met Asp Ser Val Gln Met Ala Ala Ser
 260 265 270
 Ile Gln Tyr Ser Gly Leu Asn Glu Tyr Gln Pro Leu Thr Thr Leu Phe
 275 280 285
 Phe Glu Phe Thr Gly Ser Pro Ala Ala Val Arg Glu Gln Val Glu Thr
 290 295 300
 Thr Glu Ala Ile Ala Ser Gly Asn Asn Gly Leu Gly Phe Ala Trp Ala
 305 310 315 320
 Glu Ser Pro Glu Asp Arg Thr Arg Leu Trp Lys Ala Arg His Asp Ala
 325 330 335
 Tyr Trp Ala Ala Lys Ala Ile Val Pro Asp Ala Arg Val Ile Ser Thr
 340 345 350

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Asp Cys Ile Val Pro Ile Ser Arg Leu Gly Glu Leu Ile Glu Gly Val
 355 360 365

His Arg Asp Ile Glu Ala Ser Gly Leu Arg Ala Pro Leu Leu Gly His
 370 375 380

Val Gly Asp Gly Asn Phe His Thr Leu Ile Ile Thr Asp Asp Thr Pro
 385 390 395 400

Glu Gly His Gln Gln Ala Leu Asp Leu Asp Arg Lys Ile Val Ala Arg
 405 410 415

Ala Leu Ser Leu Asn Gly Ser Cys Ser Gly Glu His Gly Val Gly Met
 420 425 430

Gly Lys Leu Glu Phe Leu Glu Thr Glu His Gly Pro Gly Ser Leu Ser
 435 440 445

Val Met Arg Ala Leu Lys Asn Thr Met Asp Pro His His Ile Leu Asn
 450 455 460

Pro Gly Lys Leu Leu Pro Pro Gly Ala Val Tyr Thr Gly
 465 470 475

<210> SEQ ID NO 39
 <211> LENGTH: 4689
 <212> TYPE: DNA
 <213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 39

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtcctt    180
tattagcctt gatagtgtcg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg    300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatatat cttttagtat aacctgaaa tttgcctat agaaatctag ggtttctgtg    480
tggtttccgg gtgagtcata cggtttttt gaatttcttt ttttgcagtt gtctctatca    540
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aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcaggggaatc    660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa    720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa    780
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aataattaat agtagtgatt ttccctaactt tatttagtca aaaaattggc cttttaattc    960
tgctgtaacc cgtacatgcc caaaataggg ggccgggttac acagaatata taacatcata    1020
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gcaattgacc tacgcatgta tctatctcat tttcttacac cttctattac cttctgtctt    1320
ctctgatttg gaaaaagctg aaaaaaagg ttgaaaccag ttcctgaaa ttattcccct    1380
attgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc    1440
    
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cagatacgcc	tcaacaggca	caggcagcac	cgacagcagc	agtcagtggt	taaaatactc	1680
tgatcatgcc	tcttcagcta	ctctattcgg	ttatttggtc	gctaagaacc	tctattctag	1740
ggagactaag	gaggacctga	tagagaaact	ggaaatggtc	aaaagatcg	acccagtaaa	1800
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gtgattaatt	aaacaggccc	cttttccttt	gtcgatatca	tgtaattagt	tatgtcacgc	3360
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gaagtctagg	tcctatttta	tttttttata	gttatgttag	tattaagaac	gttatttata	3480
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ttgatgacaa	tacagacgat	gataacaaac	cgaagttatc	tgatgtagaa	aaggattaga	3720
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ccaagctatt taatatcatg cacgaaaagc aaacaaactt gtgtgcttca ttggatgttc 4200
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cattatccgc caagtacaat tttttactct tcgaagacag aaaatttgcg gacattggta 4380
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ttatgacac 4689
    
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<210> SEQ ID NO 40
<211> LENGTH: 587
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae
    
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<400> SEQUENCE: 40

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Pro Arg Gly Arg Leu Val Arg Arg Ser Cys Tyr Arg Tyr Ala Ser Thr
          20          25          30
Gly Thr Gly Ser Thr Asp Ser Ser Ser Gln Trp Leu Lys Tyr Ser Val
          35          40          45
Ile Ala Ser Ser Ala Thr Leu Phe Gly Tyr Leu Phe Ala Lys Asn Leu
          50          55          60
Tyr Ser Arg Glu Thr Lys Glu Asp Leu Ile Glu Lys Leu Glu Met Val
 65          70          75          80
Lys Lys Ile Asp Pro Val Asn Ser Thr Leu Lys Leu Ser Ser Leu Asp
          85          90          95
Ser Pro Asp Tyr Leu His Asp Pro Ala Lys Ile Asp Lys Val Val Glu
          100          105          110
Asp Leu Lys Gln Val Leu Gly Asn Lys Pro Glu Asn Tyr Ser Asp Ala
          115          120          125
Lys Ser Asp Leu Asp Ala His Ser Asp Thr Tyr Phe Asn Thr His His
          130          135          140
Pro Ser Leu Glu Gln Arg Pro Arg Ile Ile Leu Phe Pro His Thr Thr
          145          150          155          160
Glu Glu Val Ser Lys Ile Leu Lys Ile Cys His Asp Asn Asn Met Pro
          165          170          175
Val Val Pro Phe Ser Gly Gly Thr Ser Leu Glu Gly His Phe Leu Pro
          180          185          190
Thr Arg Ile Gly Asp Thr Ile Thr Val Asp Leu Ser Lys Phe Met Asn
          195          200          205
    
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Asn Val Val Lys Phe Asp Lys Leu Asp Leu Asp Ile Thr Val Gln Ala
 210 215 220

Gly Leu Pro Trp Glu Asp Leu Asn Asp Tyr Leu Ser Asp His Gly Leu
 225 230 235 240

Met Phe Gly Cys Asp Pro Gly Pro Gly Ala Gln Ile Gly Gly Cys Ile
 245 250 255

Ala Asn Ser Cys Ser Gly Thr Asn Ala Tyr Arg Tyr Gly Thr Met Lys
 260 265 270

Glu Asn Ile Ile Asn Met Thr Ile Val Leu Pro Asp Gly Thr Ile Val
 275 280 285

Lys Thr Lys Lys Arg Pro Arg Lys Ser Ser Ala Gly Tyr Asn Leu Asn
 290 295 300

Gly Leu Phe Val Gly Ser Glu Gly Thr Leu Gly Ile Val Thr Glu Ala
 305 310 315 320

Thr Val Lys Cys His Val Lys Pro Lys Ala Glu Thr Val Ala Val Val
 325 330 335

Ser Phe Asp Thr Ile Lys Asp Ala Ala Ala Cys Ala Ser Asn Leu Thr
 340 345 350

Gln Ser Gly Ile His Leu Asn Ala Met Glu Leu Leu Asp Glu Asn Met
 355 360 365

Met Lys Leu Ile Asn Ala Ser Glu Ser Thr Asp Arg Cys Asp Trp Val
 370 375 380

Glu Lys Pro Thr Met Phe Phe Lys Ile Gly Gly Arg Ser Pro Asn Ile
 385 390 395 400

Val Asn Ala Leu Val Asp Glu Val Lys Ala Val Ala Gln Leu Asn His
 405 410 415

Cys Asn Ser Phe Gln Phe Ala Lys Asp Asp Asp Glu Lys Leu Glu Leu
 420 425 430

Trp Glu Ala Arg Lys Val Ala Leu Trp Ser Val Leu Asp Ala Asp Lys
 435 440 445

Ser Lys Asp Lys Ser Ala Lys Ile Trp Thr Thr Asp Val Ala Val Pro
 450 455 460

Val Ser Gln Phe Asp Lys Val Ile His Glu Thr Lys Lys Asp Met Gln
 465 470 475 480

Ala Ser Lys Leu Ile Asn Ala Ile Val Gly His Ala Gly Asp Gly Asn
 485 490 495

Phe His Ala Phe Ile Val Tyr Arg Thr Pro Glu Glu His Glu Thr Cys
 500 505 510

Ser Gln Leu Val Asp Arg Met Val Lys Arg Ala Leu Asp Ala Glu Gly
 515 520 525

Thr Cys Thr Gly Glu His Gly Val Gly Ile Gly Lys Arg Glu Tyr Leu
 530 535 540

Leu Glu Glu Leu Gly Glu Ala Pro Val Asp Leu Met Arg Lys Ile Lys
 545 550 555 560

Leu Ala Ile Asp Pro Lys Arg Ile Met Asn Pro Asp Lys Ile Phe Lys
 565 570 575

Thr Asp Pro Asn Glu Pro Ala Asn Asp Tyr Arg
 580 585

<210> SEQ ID NO 41
 <211> LENGTH: 4527
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:

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Saccharomyces cerevisiae and *Issatchenkia orientalis*.

<400> SEQUENCE: 41

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcacct   180
tattagcctt gatagtgtcg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg   240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg   300
gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag   360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc   420
aataatata cttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg   480
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atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat   600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcagggaatc   660
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<211> LENGTH: 533

<212> TYPE: PRT

<213> ORGANISM: *Issatchenkia orientalis*

<400> SEQUENCE: 42

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 35 40 45
 Pro Ile Thr Ala Ala Pro Pro Leu Gln Tyr Gly Asp Val Ser Lys Ala
 50 55 60
 Ile Ser Glu Ile Thr Ser Lys Leu Gly Pro Ser Lys Ile Thr Phe Ser
 65 70 75 80
 Glu Ser Glu Ile Leu His His Ser Asp Ser Ser Phe Ser Thr Asp His
 85 90 95
 Ala Lys Pro His Glu Arg Pro His Ala Val Val Tyr Pro Glu Ser Thr
 100 105 110
 Glu Asp Val Ser Glu Val Leu Lys Ile Cys His Thr Leu Lys Val Pro
 115 120 125
 Val Val Pro Phe Thr Gly Gly Thr Ser Leu Glu Gly His Phe Ile Ser
 130 135 140
 Thr Arg Asn Gly Ile Ser Val Asp Leu Ser Arg Met Asn Lys Val Leu
 145 150 155 160
 Ala Ile His Pro Glu Asp Leu Asp Ala Val Val Gln Pro Ala Val Gly
 165 170 175
 Trp Glu Glu Leu Arg Asp Thr Leu Ser Asp Tyr Asn Leu Leu Phe Gly
 180 185 190
 Pro Asp Pro Gly Pro Gly Ala Cys Ile Gly Gly Met Val Ala Thr Ser
 195 200 205
 Cys Ser Gly Thr Asn Ala Ala Arg Tyr Gly Thr Met Arg Glu Asn Val
 210 215 220
 Val Ser Val Lys Val Val Leu Pro Asp Gly Thr Ile Val Lys Thr Lys
 225 230 235 240
 Arg Arg Pro Arg Lys Ser Ser Ala Gly Tyr Asn Leu Thr Asn Leu Phe
 245 250 255
 Ile Gly Ser Glu Gly Thr Leu Gly Ile Val Thr Glu Val Thr Leu Lys
 260 265 270
 Leu Asn Val Lys Pro Lys Tyr Glu Ser Val Ala Leu Val Ser Phe Asp
 275 280 285
 Lys Leu Lys Asp Ala Ala Gly Ser Val Thr Ser Phe Val Gln Glu Gly
 290 295 300
 Leu Gln Leu Asn Ala Ile Glu Leu Leu Asp Asp Lys Met Val His Phe
 305 310 315 320
 Val Asn Glu Ser Gly Glu Thr Asp Leu Lys Tyr Asp Glu Leu Pro Thr
 325 330 335
 Leu Leu Leu Lys Ile Gly Gly Ser Ser Pro Glu Ser Val Asn Leu Leu
 340 345 350
 Ile Lys Thr Val Glu Arg Ile Val Asn Ala Asn Asn Ala Lys Thr Phe
 355 360 365
 Lys Phe Ala Ser Asn Glu Glu Glu Lys Tyr Gln Leu Trp Asn Ala Arg
 370 375 380
 Lys Thr Ala Leu Trp Ser Thr Ile Glu Tyr Gly Arg Arg Asn Ile Asp

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385	390	395	400
Lys Asp Ile Gln Val Trp Thr Thr Asp Val Ala Val Pro Met Ser Lys	405	410	415
Phe Val Glu Ala Val Glu Glu Thr Gln Lys Glu Ile Glu Glu Ser Asp	420	425	430
Leu Leu Ala Ser Ile Val Gly His Ala Gly Asp Gly Asn Tyr His Thr	435	440	445
Val Ile Leu Phe Lys Glu Glu Gln Arg Ala Leu Ala Ala Lys Leu Val	450	455	460
Ser Asn Met Val Asp Arg Ala Leu Ala Phe Asp Gly Thr Val Ser Gly	465	470	475
Glu His Gly Ile Gly Val Gly Lys Lys Glu Phe Leu Ile Asp Glu Val	485	490	495
Gly Gln Glu Ser Val Asp Leu Met Arg Lys Ile Lys Phe Ser Ile Asp	500	505	510
Pro His Lys Ile Leu Asn Pro Asp Lys Val Phe Ser Ile Asp Pro Val	515	520	525
Asn Asp Arg Ser Ile	530		

<210> SEQ ID NO 43
 <211> LENGTH: 4506
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Issatchenkia orientalis.

<400> SEQUENCE: 43

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attaattaga acaagaactt atttagaaca cgttcactct gagtaagaac tcttgcctct    180
tattagcctt gatagtgtctg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg    300
gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatatat cttttagtat aaccctgaaa tttgcctctat agaaatctag ggtttctgtg    480
tgggttccgg gtgagtcata cggccttttt gaatttcttt ttttgcagtt gtctctatca    540
atgaaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat    600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaacaggaa tcagggaatc    660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa    720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa    780
agtttatttc agagttcttc agacttctta actcctgtaa aaacaaaaaa aaaaaaaggc    840
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taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccaac    1140
catcagttca taggtccatt ctcttagcgc aactacacag aacaggggca caaacaggca    1200
    
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atgtgactaa	taagtatata	aagacggtag	gtattgattg	taattctgta	aatctatttc	1440
ttaaacttct	taaattctac	ttttatagtt	agtctttttt	ttagtttaaa	acaccaagaa	1500
cttagtttct	aataaacaca	cataaacaaa	caaatctaga	atgtttgcat	tagcttctat	1560
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ccgtggattt	gcgttgactg	ctgaaaaata	tccttcagtt	aagcgtggag	actacgcaa	1680
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caaattttct	ttttttttct	gtacaaaacgc	gtgtacgcat	gtaacgggca	gacggccggc	3360
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atgacaatc	agacgatgat	aacaaaccga	agttatctga	tgtagaaaag	gattagagat	3540

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cagtcaaatt gcagtactct gcgggtgat acagaatagc agaatgggca gacattacga 4260
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tgacac 4506
    
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<210> SEQ ID NO 44
<211> LENGTH: 526
<212> TYPE: PRT
<213> ORGANISM: Issatchenkia orientalis
    
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<400> SEQUENCE: 44

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Met Phe Ala Leu Ala Ser Ile Ser Arg Leu Gly Arg Ala Ser Ala Ser
 1             5             10             15
Ala Arg Ala Arg Ala Ser Ala Ala Leu Ser Ile Arg Gly Phe Ala Leu
          20             25             30
Thr Ala Glu Lys Tyr Pro Ser Val Lys Arg Gly Asp Tyr Ala Lys Leu
          35             40             45
Thr Asp Glu Asp Leu Gly Lys Phe His Ser Ile Leu Asn Pro Asn Gln
          50             55             60
Ile Ile Thr Asp Ala Ala Asp Leu Ala Ala Phe Asn Gln Asp Trp Met
          65             70             75             80
Lys Lys Tyr Ala Gly Gln Ser Gln Leu Val Leu Lys Pro Lys Thr Thr
          85             90             95
Gln Gln Val Ser Gln Ile Leu Lys Tyr Cys Asn Glu Arg Lys Leu Ala
          100            105            110
Val Val Pro Gln Gly Gly Asn Thr Gly Leu Val Gly Gly Ser Val Pro
          115            120            125
Val Phe Asp Glu Ile Val Ile Ser Met Ala Gly Met Asn Gln Ile Arg
          130            135            140
Ser Phe Asp Glu Asn Ser Gly Thr Leu Lys Val Asp Ala Gly Val Ile
          145            150            155            160
Leu Glu Asn Ala Asp Asn Tyr Leu Ala Glu Arg Gly Tyr Ile Phe Pro
          165            170            175
Leu Asp Leu Gly Ala Lys Gly Ser Cys Phe Val Gly Gly Asn Val Ala
          180            185            190
Thr Asn Ala Gly Gly Leu Arg Leu Leu Arg Phe Gly Ser Leu His Gly
    
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195					200					205					
Ser	Val	Leu	Gly	Leu	Glu	Val	Val	Leu	Ala	Asp	Gly	Thr	Ile	Val	Asp
210					215					220					
Ser	Met	His	Ser	Leu	Arg	Lys	Asp	Asn	Thr	Gly	Phe	Asp	Leu	Lys	Gln
225					230					235					240
Leu	Phe	Ile	Gly	Ser	Glu	Gly	Thr	Leu	Gly	Ile	Ile	Thr	Gly	Val	Ser
				245					250					255	
Ile	Leu	Cys	Pro	Ala	Arg	Pro	Lys	Ala	Val	Asn	Ile	Ala	Phe	Leu	Gly
				260					265					270	
Leu	Glu	Ser	Tyr	Glu	His	Val	Ile	Gln	Cys	Phe	Lys	Glu	Ala	Arg	Ser
				275					280					285	
Asp	Leu	Gly	Glu	Ile	Leu	Ser	Ala	Phe	Glu	Phe	Met	Asp	Arg	Asp	Ser
290					295					300					
Gln	Leu	Val	Ala	Ser	Lys	Phe	Leu	Lys	Thr	Thr	His	Pro	Leu	Cys	Gln
305					310					315					320
Glu	Asp	Glu	Val	Ala	Glu	Ala	Ser	Val	Pro	Glu	Tyr	Pro	Phe	Tyr	Val
				325					330					335	
Leu	Leu	Glu	Thr	Ser	Gly	Ser	Asn	Lys	Asp	His	Asp	Asp	Glu	Lys	Leu
				340					345					350	
Glu	Lys	Phe	Leu	Glu	Lys	Val	Met	Glu	Asn	Glu	Val	Val	Val	Asp	Gly
				355					360					365	
Thr	Val	Ser	Gln	Asp	Glu	Ala	Gln	Leu	Lys	Thr	Leu	Trp	Thr	Trp	Arg
370					375					380					
Glu	Gly	Ile	Ala	Glu	Ala	Ser	Gln	Gln	Phe	Gly	Gly	Val	Tyr	Lys	Tyr
385					390					395					400
Asp	Val	Ser	Leu	Pro	Leu	Asp	Ser	Leu	Tyr	Lys	Leu	Val	Glu	Glu	Thr
				405					410					415	
Gln	Glu	Arg	Leu	Lys	Ser	Phe	Ser	Leu	Ala	Ser	Thr	Glu	Asp	Glu	Ser
				420					425					430	
Lys	Pro	Val	Tyr	Glu	Ala	Ile	Gly	Tyr	Gly	His	Ile	Gly	Asp	Gly	Asn
				435					440					445	
Leu	His	Leu	Asn	Val	Ala	Val	Arg	Glu	Tyr	Asn	Lys	Glu	Val	Glu	Lys
450					455					460					
Ala	Leu	Glu	Pro	Phe	Val	Tyr	Glu	Phe	Ile	Glu	Lys	His	Asn	Gly	Ser
465					470					475					480
Ile	Ser	Ala	Glu	His	Gly	Leu	Gly	Phe	Gln	Lys	Lys	Asn	Tyr	Ile	Gly
				485					490					495	
Tyr	Ser	Lys	Ser	Glu	Val	Glu	Ile	Lys	Met	Met	Lys	Glu	Leu	Lys	Asn
				500					505					510	
His	Tyr	Asp	Pro	Asn	Gly	Ile	Leu	Asn	Pro	Tyr	Lys	Tyr	Ile		
				515					520					525	

<210> SEQ ID NO 45
 <211> LENGTH: 4629
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Issatchenkia orientalis.

<400> SEQUENCE: 45
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 gtatttccga gatacgatta ctccagttcc tcttacaaga aatgcataaa aatagttaca 120
 attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcctct 180

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tattagcctt gatagtgctg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg	240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg	300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag	360
attccaaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc	420
aataatata ctttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg	480
tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgcagtt gtctctatca	540
atgaaaattt cgaggaagac gataaggta agataagtag ataagagaat gatacgagat	600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcagggaatc	660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa	720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa	780
agtttatctc agagttcttc agactctta actcctgtaa aaacaaaaaa aaaaaaggc	840
atagcactcg agatctccc agtttatcat tatcaatact gccatttcaa agaatacgt	900
aataattaat agtagtgatt ttcctaactt tatttagtca aaaaattggc cttttaattc	960
tgctgtaacc cgtacatgcc caaaataggg ggcgggttac acagaatata taacatcata	1020
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taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccaac	1140
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ctctgatttg gaaaaagctg aaaaaaagg ttgaaaccag ttccctgaaa ttattcccct	1380
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cttagtttgc aataaacaca cataaacaaa caaatctaga atgataatgt tgccgttgtc	1560
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aattgtcccc tacactgggt gcacctcatt ggagggacat tatattccaa ctaggaaggg	2160
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tgcaagttgcc aaaatcgtcc agtccggtt acaagtcaat gctttgaaa tgttgatc	2580

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tcagagtaag gaaaaattgg gtcaagatgt tcaactgtgg acaaccgacg tggcagttcc	2880
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tagctactgg agaataact aagggtactg ttgacattgc gaagagcagc aaagattttg	4560
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ttatgacac	4629

<210> SEQ ID NO 46

<211> LENGTH: 567

<212> TYPE: PRT

<213> ORGANISM: *Issatchenkia orientalis*

<400> SEQUENCE: 46

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Met Ile Met Leu Pro Leu Ser Arg Ala Ala Val His Leu Ser Arg Thr
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 Arg His Ser Ser Leu Val Ser Leu Arg His Ala Leu Ala Ile Pro Gln
 20 25 30
 Arg Val Gln Tyr Ser His His Arg His Tyr Ser Ala Asn Thr Glu Lys
 35 40 45
 Thr Ala Lys Thr Gly Ser Glu Lys Asp Pro Lys Ser Gln Ala Arg Lys
 50 55 60
 Asp Leu Gly Lys Ser Phe Leu Ser Thr Thr Ala Ile Leu Phe Ile Gly
 65 70 75 80
 Ala Leu Val Gly Thr Ser Trp Ala Ser Phe Gln Leu Cys Lys Asn Pro
 85 90 95
 Pro Glu Phe Leu Phe Pro His Thr Ser Thr Phe Pro Leu Lys Asp Ala
 100 105 110
 Met Pro Pro Leu Tyr Gly Asp Ser Gln Lys Val Ile Asp Glu Leu Lys
 115 120 125
 Pro Leu Leu Arg Glu Asp Gln Ile Thr Ile Ser Lys Ser Glu Leu Asp
 130 135 140
 Thr His Ser Asp Ala Tyr Phe Ser Thr Asp His Pro Ser Asp Glu Glu
 145 150 155 160
 Arg Pro Asp Ala Ile Val Tyr Pro Glu Ser Thr Glu Gln Val Ser Gln
 165 170 175
 Ile Leu Lys Ile Ala His Asn His His Val Pro Ile Val Pro Tyr Thr
 180 185 190
 Gly Gly Thr Ser Leu Glu Gly His Tyr Ile Pro Thr Arg Lys Gly Ile
 195 200 205
 Cys Val Asp Leu Ser Arg Leu Asn Lys Val Leu Ala Leu His Lys Glu
 210 215 220
 Asp Leu Asp Ile Val Val Gln Pro Ala Val Gly Trp Glu Asp Leu Asp
 225 230 235 240
 Glu Phe Leu Arg Pro Gln Gly Leu Leu Phe Gly Pro Asp Pro Gly Pro
 245 250 255
 Gly Ala Cys Ile Gly Gly Met Cys Gly Thr Ser Cys Ser Gly Thr Asn
 260 265 270
 Ala Ala Arg Tyr Gly Thr Met Arg Glu Asn Val Val Ser Leu Thr Val
 275 280 285
 Val Leu Ala Asp Gly Thr Leu Gly Ile Val Thr Glu Ala Thr Leu Lys
 290 295 300
 Leu His Val Ala Pro Lys Phe Glu Asn Val Ala Val Val Asn Phe Pro
 305 310 315 320
 Thr Leu Arg Asp Ala Ala Asn Ala Val Ala Lys Ile Val Gln Ser Gly
 325 330 335
 Leu Gln Val Asn Ala Leu Glu Met Leu Asp Thr Glu Met Met Gln Phe
 340 345 350
 Val Asn Gln Ser Gly Asn Val Thr Lys Lys Tyr Ala Glu Ser Pro Thr
 355 360 365
 Leu Met Met Lys Leu Gly Gly Asn Ser Lys Thr Val Val Asp Ala Leu
 370 375 380
 Thr Lys Asp Val Lys Ser Ile Cys Thr Glu Cys Asn Ile Gly Arg Lys
 385 390 395 400
 Asp Phe Lys Phe Ala Glu Asp Glu Glu Glu Lys Glu Glu Leu Trp Thr
 405 410 415
 Ala Arg Lys Val Ala Leu Trp Ser Thr Leu Asp Ala Gly Lys Glu Lys

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420	425	430
Leu Gly Gln Asp Val Gln Leu Trp Thr Thr Asp Val Ala Val Pro Ile		
435	440	445
Ser Lys Leu Val Asp Ser Leu Glu Asn Thr Arg Arg Leu Leu Asn Glu		
450	455	460
Ala Gly Phe Met Ser Ser Ile Val Ser His Ala Gly Asp Gly Asn Tyr		
465	470	475
His Ala Phe Ile Val Tyr Asp Lys Lys Asp Tyr Val Lys Ala Ala Asn		
485	490	495
Val Val Lys Glu Met Val His His Ala Leu Glu Leu Asp Gly Thr Ala		
500	505	510
Thr Gly Glu His Gly Ile Gly Ile Gly Lys Arg Glu Phe Leu Arg Glu		
515	520	525
Glu Leu Gly Asp Ala Pro Ile Asp Leu Met Arg Lys Ile Lys Phe Ala		
530	535	540
Leu Asp Pro Leu Leu Leu Leu Asn Pro Asp Lys Val Phe Lys Ile Asp		
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Pro Glu Asp Thr Arg Leu Ser		
565		

<210> SEQ ID NO 47
 <211> LENGTH: 4199
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Lactobacillus plantarum.

<400> SEQUENCE: 47

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcacct    180
tattagcctt gatagtgctg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg    300
gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatatat cttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg    480
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<210> SEQ ID NO 48

<211> LENGTH: 424

<212> TYPE: PRT

<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 48

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20          25          30
Tyr His Asn Lys Leu Ser Glu Gln Glu Thr Val Glu Lys Ser Leu Asp
35          40          45
Asn Pro Ile Gly Ser Asp Lys Leu Glu Glu Leu Ala Arg Gly Lys His
50          55          60
Asn Ile Val Ile Ile Ser Ser Asp His Thr Arg Pro Val Pro Ser His
65          70          75          80
Ile Ile Thr Pro Ile Leu Leu Arg Arg Leu Arg Ser Val Ala Pro Asp
85          90          95
Ala Arg Ile Arg Ile Leu Val Ala Thr Gly Phe His Arg Pro Ser Thr
100         105         110
His Glu Glu Leu Val Asn Lys Tyr Gly Glu Asp Ile Val Asn Asn Glu
115        120        125
Glu Ile Val Met His Val Ser Thr Asp Asp Ser Ser Met Val Lys Ile
130        135        140
Gly Gln Leu Pro Ser Gly Gly Asp Cys Ile Ile Asn Lys Val Ala Ala
145        150        155        160
Glu Ala Asp Leu Leu Ile Ser Glu Gly Phe Ile Glu Ser His Phe Phe
165        170        175
Ala Gly Phe Ser Gly Gly Arg Lys Ser Val Leu Pro Gly Ile Ala Ser
180        185        190
Tyr Lys Thr Ile Met Ala Asn His Ser Gly Glu Phe Ile Asn Ser Pro
195        200        205
Lys Ala Arg Thr Gly Asn Leu Met His Asn Ser Ile His Lys Asp Met
210        215        220
Val Tyr Ala Ala Arg Thr Ala Lys Leu Ala Phe Ile Ile Asn Val Val
225        230        235        240
Leu Asp Glu Asp Lys Lys Ile Ile Gly Ser Phe Ala Gly Asp Met Glu
245        250        255
Ala Ala His Lys Val Gly Cys Asp Phe Val Lys Glu Leu Ser Ser Val

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260				265				270							
Pro	Ala	Ile	Asp	Cys	Asp	Ile	Ala	Ile	Ser	Thr	Asn	Gly	Gly	Tyr	Pro
	275						280					285			
Leu	Asp	Gln	Asn	Ile	Tyr	Gln	Ala	Val	Lys	Gly	Met	Thr	Ala	Ala	Glu
	290					295					300				
Ala	Thr	Asn	Lys	Glu	Gly	Gly	Thr	Ile	Ile	Met	Val	Ala	Gly	Ala	Arg
	305				310					315					320
Asp	Gly	His	Gly	Gly	Glu	Gly	Phe	Tyr	His	Asn	Leu	Ala	Asp	Val	Asp
			325						330					335	
Asp	Pro	Lys	Glu	Phe	Leu	Asp	Gln	Ala	Ile	Asn	Thr	Pro	Arg	Leu	Lys
		340					345						350		
Thr	Ile	Pro	Asp	Gln	Trp	Thr	Ala	Gln	Ile	Phe	Ala	Arg	Ile	Leu	Val
		355					360					365			
His	His	His	Val	Ile	Phe	Val	Ser	Asp	Leu	Val	Asp	Pro	Asp	Leu	Ile
	370					375					380				
Thr	Asn	Met	His	Met	Glu	Leu	Ala	Lys	Thr	Leu	Asp	Glu	Ala	Met	Glu
	385				390					395					400
Lys	Ala	Tyr	Ala	Arg	Glu	Gly	Gln	Ala	Ala	Lys	Val	Thr	Val	Ile	Pro
			405						410					415	
Asp	Gly	Leu	Gly	Val	Ile	Val	Lys								
		420													

<210> SEQ ID NO 49
 <211> LENGTH: 5084
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Aspergillus nidulans.

<400> SEQUENCE: 49

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcctct	180
tattagcctt gatagtgctg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg	240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg	300
gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag	360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc	420
aataatatat cttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg	480
tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgcagtt gtctctatca	540
atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat	600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaacccgaa tcagggaatc	660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa	720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa	780
agtttatttc agagttcttc agacttctta actcctgtaa aaacaaaaaa aaaaaaggc	840
atagcactcg agatctcccg agtttatcat tatcaatact gccatttcaa agaatacgt	900
aataattaat agtagtgatt ttccctaact tattttagtc aaaaattggc cttttaattc	960
tgctgtaacc cgtacatgcc caaaataggg ggccgggttac acagaatata taacatcata	1020
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<210> SEQ ID NO 50

<211> LENGTH: 5343

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae, Kluyveromyces lactis and Aspergillus
 nidulans.

<400> SEQUENCE: 50

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<210> SEQ ID NO 51
<211> LENGTH: 562
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae
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<400> SEQUENCE: 51
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35 40 45
Asn Ile Ala Ser Phe Asp Arg Glu Arg Val Pro Glu Arg Val Val His
50 55 60
Ala Lys Gly Gly Gly Cys Arg Leu Glu Phe Glu Leu Thr Asp Ser Leu
65 70 75 80
Ser Asp Ile Thr Tyr Ala Ala Pro Tyr Gln Asn Val Gly Tyr Lys Cys
85 90 95
Pro Gly Leu Val Arg Phe Ser Thr Val Gly Gly Glu Ser Gly Thr Pro
100 105 110
Asp Thr Ala Arg Asp Pro Arg Gly Val Ser Phe Lys Phe Tyr Thr Glu
115 120 125
Trp Gly Asn His Asp Trp Val Phe Asn Asn Thr Pro Val Phe Phe Leu
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145 150 155 160
Pro Gln Ser His Leu Asn Gln Phe Gln Asp Thr Thr Ile Tyr Trp Asp
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Tyr Leu Thr Leu Asn Pro Glu Ser Ile His Gln Ile Thr Tyr Met Phe
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Gly Asp Arg Gly Thr Pro Ala Ser Trp Ala Ser Met Asn Ala Tyr Ser
195 200 205
Gly His Ser Phe Ile Met Val Asn Lys Glu Gly Lys Asp Thr Tyr Val
210 215 220
Gln Phe His Val Leu Ser Asp Thr Gly Phe Glu Thr Leu Thr Gly Asp
225 230 235 240
Lys Ala Ala Glu Leu Ser Gly Ser His Pro Asp Tyr Asn Gln Ala Lys
245 250 255
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260 265 270
Val Gln Thr Met Thr Pro Glu Gln Ala Thr Lys Phe Arg Tyr Ser Val
275 280 285
Asn Asp Leu Thr Lys Ile Trp Pro His Lys Glu Phe Pro Leu Arg Lys
290 295 300
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305 310 315 320
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Asn	Ala	Lys	Gly	Glu	Phe	Glu	Lys	Gly	Phe	Tyr	Asp	Tyr	Gly	Lys	Val
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Gln	Met	Met	Val	Asp	Ala	Glu	His	Arg	Leu	Asp	Ala	Glu	Glu	Lys	Gly
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Asp	Phe	Glu	Asn	Leu	Glu	Arg	Val	Asp	Ser	Glu	Gly	Lys	Gln	Met	Asp
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Asn	Phe	Val	Glu	Glu	Val	Ala	Glu	Pro	Glu	Gly	Val	Tyr	Thr	Gly	Ser
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<210> SEQ ID NO 53
 <211> LENGTH: 4656
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

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<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Saccharomyces kluyveri.

<400> SEQUENCE: 53

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<400> SEQUENCE: 54
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Met Pro Gln Asp Leu Val Cys Ser Pro Leu Thr Pro Gly Glu Thr Pro
1 5 10 15
Asp Asp Ile Val Arg Lys Glu Glu Leu Arg Ser Arg Leu Pro Ser Leu
20 25 30
Asp Ala Leu Val Asn Leu Tyr Asp Phe Glu Tyr Leu Ala Ser Gln Ile
35 40 45
Leu Thr Lys Gln Ala Trp Ser Tyr Tyr Ser Ser Ala Ala Asp Asp Glu
50 55 60
Val Thr His Arg Glu Asn His Ala Ala Tyr His Arg Ile Phe Phe Lys
65 70 75 80
Pro Lys Val Leu Val Asp Val Arg Glu Val Asp Thr Ser Thr Lys Ile
85 90 95
Leu Gly Gln Asp Val Pro Val Pro Phe Tyr Val Ser Ala Thr Ala Leu
100 105 110
Cys Lys Leu Gly Asn Pro Lys Glu Gly Glu Lys Asp Val Ala Arg Gly
115 120 125
Cys Gly Gln Gly Pro Ile Lys Cys Pro Gln Met Ile Ser Thr Leu Ala
130 135 140
Ser Cys Ser Leu Gln Glu Ile Val Glu Ala Ala Pro Ser Lys Glu Gln
145 150 155 160
Ile Gln Trp Phe Gln Leu Tyr Val Asn Thr Asp Arg Lys Ile Thr Asp
165 170 175
Asp Leu Ile Lys Gln Val Glu Lys Leu Gly Leu Lys Ala Ile Phe Val
180 185 190
Thr Val Asp Ala Pro Ser Leu Gly Arg Arg Glu Lys Asp Met Lys Val
195 200 205
Lys Phe Thr Asn Ser Asn Gly Ala Lys Ala Met Glu Lys Thr Lys Val
210 215 220
Lys Glu Ser Lys Gly Ala Ser Ala Ala Leu Ser Thr Phe Ile Asp Pro
225 230 235 240
Ala Leu Ser Trp Asp Asp Ile Val Glu Ile Lys Lys Lys Thr Asn Leu
245 250 255
Pro Ile Val Ile Lys Gly Val Gln Arg Val Glu Asp Val Leu Lys Ala
260 265 270
Ala Glu Val Gly Ala Ala Gly Val Val Leu Ser Asn His Gly Gly Arg
275 280 285
Gln Leu Asp Phe Ser Arg Ala Pro Ile Glu Val Leu Ala Glu Ser Val
290 295 300
Pro Val Leu Lys Glu Arg Asn Leu Ser Asp Lys Leu Glu Ile Phe Ile
305 310 315 320
Asp Gly Gly Val Arg Arg Gly Asn Asp Val Leu Lys Ala Leu Cys Leu
325 330 335
Gly Ala Lys Gly Val Gly Leu Gly Arg Pro Phe Leu Tyr Ala Asn Ser
340 345 350
Cys Tyr Gly Lys Asp Gly Val Gln Lys Ala Ile Glu Met Leu Thr Glu
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355	360	365
Glu Ile Gln Met Ser Met Arg Leu Leu Gly Val Thr Lys Ile Glu Asp 370 375 380		
Leu Lys Pro Glu Leu Leu Asp Met Ser Ser Phe Ser Gly Arg Thr Val 385 390 395 400		
Asn Val Pro Gln Asp Ser Leu Tyr Arg Ser Val Tyr Glu Lys Ser Glu 405 410 415		
Thr Val Gln Phe Leu Asp Glu 420		

<210> SEQ ID NO 55
 <211> LENGTH: 4704
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Saccharomyces bayanus.

<400> SEQUENCE: 55

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtccct    180
tattagcctt gatagtgtg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg    300
gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatatat cttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg    480
tgggttccgg gtgagtcata cggccttttt gaatttcttt ttttgcagtt gtctctatca    540
atgaaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat    600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaacggaa tcagggaatc    660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgatatatc tggaacagaa    720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa    780
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aataattaat agtagtgatt ttoctaactt tatttagtca aaaaattggc cttttaattc    960
tgctgtaacc cgtacatgcc caaaataggg ggcgggttac acagaatata taacatcata    1020
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taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccac    1140
catcagttca taggtccatt ctcttagcgc aactacacag aacaggggca caaacaggca    1200
aaaaacgggc acaacctcaa tggagtgatg caacctgctt ggagtaaatg atgacacaag    1260
gcaattgacc tacgcatgta tctatctcat tttcttacac cttctattac cttctgtct    1320
ctctgatttg gaaaaagctg aaaaaaagg ttgaaaccag ttcctgaaa ttattcccct    1380
atttgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc    1440
ttaaacttct taaattctac ttttatagtt agtctttttt ttagttttaa acaccaagaa    1500
cttagtttcg aataaacaca cataaacaaa caaatctaga atgttaaaat acaaatctct    1560
gctgagaatc tcgagagtct ccgaggcagc cgtctctaga acgtocagga ctagattaaa    1620
    
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cacagtccgc tcttacagtt ctgctgtggg caagtctaag tegtctgaga aacattccgg	1680
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aatggcttat gtaaacctggg gtagtggcca gataggtaac gacccaaaac tagacatgaa	1800
taagcagaaa atatcgctg cagaagtgc taaacacaac tcggctgacg attgctgggt	1860
cgtgatcaac ggttatgtgt atgatctgac gcggttcgtc cctaaccatc cagggtggccc	1920
ggatgttatc aaatacaatg ctgggagaga cgtcactgcc atttttgagc cattgcatgc	1980
ccccaacgtc atcgataaat acattgctcc tgagaagaag ctgggaccgc tacaggggtc	2040
catgccacca gaactggttt gcccgccata cgctccaggc gaaaccaaag aagacattgc	2100
taggaaagag caactaaaat cattaactccc ccaactggat aacattatta atctttatga	2160
tttcgagtac ttggcttctc aaactttgac taggcaagcc tgggcttact actcctctgg	2220
ttccaatgac gaagtctcgc acagggaaaa ccataatgca taccacagga ttttcttcaa	2280
gccaagatt cttgttgacg tgagcaaatg ggacatctcg acggacatgc tgggttctca	2340
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aggtagaaaag gacattgcta gaggctgtgg ccaaggcgcg acgaaagtcc cacaaatgat	2460
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catagacggt ggcgttcgtc gtggtacaga tatcctaaag gcgctgtgc tgggctgtaa	3060
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agaacggtat ttatatttca aatttttctt ttttttctgt acaaacgcgt gtacgcatgt	3540
aacgggcaga cggccggcca taactctgta taatgtatgc tatacgaagt tatggcaacg	3600
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gtgttgaaga aacatgaaat tgcccagtat tcttaacca actgcacaga aaaaaaacct 4080
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ctagtctctgt tgctgccaag ctatttaata tcatgcacga aaagcaaaca aacttgtgtg 4200
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gttacgattg gttgattatg acac 4704

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<210> SEQ ID NO 56

<211> LENGTH: 591

<212> TYPE: PRT

<213> ORGANISM: Saccharomyces bayanus

<400> SEQUENCE: 56

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20          25          30
Ser Ser Ala Val Gly Lys Ser Lys Ser Phe Glu Lys His Ser Gly Lys
35          40          45
Arg Thr Gln Ser Trp Thr Ala Leu Ser Val Gly Ala Ile Leu Ala Ala
50          55          60
Ala Gly Ser Met Ala Tyr Val Asn Trp Gly Ser Gly Gln Ile Gly Asn
65          70          75          80
Asp Pro Lys Leu Asp Met Asn Lys Gln Lys Ile Ser Pro Ala Glu Val
85          90          95
Ala Lys His Asn Ser Ala Asp Asp Cys Trp Val Val Ile Asn Gly Tyr
100         105         110
Val Tyr Asp Leu Thr Arg Phe Val Pro Asn His Pro Gly Gly Pro Asp
115        120        125
Val Ile Lys Tyr Asn Ala Gly Arg Asp Val Thr Ala Ile Phe Glu Pro
130        135        140
Leu His Ala Pro Asn Val Ile Asp Lys Tyr Ile Ala Pro Glu Lys Lys
145        150        155        160
Leu Gly Pro Leu Gln Gly Ser Met Pro Pro Glu Leu Val Cys Pro Pro
165        170        175
Tyr Ala Pro Gly Glu Thr Lys Glu Asp Ile Ala Arg Lys Glu Gln Leu
180        185        190
Lys Ser Leu Leu Pro Pro Leu Asp Asn Ile Ile Asn Leu Tyr Asp Phe
195        200        205
Glu Tyr Leu Ala Ser Gln Thr Leu Thr Arg Gln Ala Trp Ala Tyr Tyr
210        215        220
Ser Ser Gly Ser Asn Asp Glu Val Ser His Arg Glu Asn His Asn Ala
225        230        235        240
Tyr His Arg Ile Phe Phe Lys Pro Lys Ile Leu Val Asp Val Ser Lys

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245				250				255							
Val	Asp	Ile	Ser	Thr	Asp	Met	Leu	Gly	Ser	His	Val	Asp	Val	Pro	Phe
			260											270	
Tyr	Val	Ser	Ala	Thr	Ala	Leu	Cys	Lys	Leu	Gly	Asn	Pro	Leu	Glu	Gly
			275												285
Glu	Lys	Asp	Ile	Ala	Arg	Gly	Cys	Gly	Gln	Gly	Ala	Thr	Lys	Val	Pro
			290												300
Gln	Met	Ile	Ser	Thr	Leu	Ala	Ser	Cys	Ser	Pro	Glu	Glu	Ile	Ile	Ser
															320
Ala	Ala	Pro	Ser	Asp	Glu	Gln	Ile	Gln	Trp	Tyr	Gln	Leu	Tyr	Val	Asn
															335
Ser	Asp	Arg	Lys	Ile	Thr	Asp	Glu	Leu	Val	Lys	Ser	Val	Glu	Lys	Leu
															350
Gly	Val	Lys	Ala	Leu	Phe	Val	Thr	Val	Asp	Ala	Pro	Ser	Leu	Gly	Gln
															365
Arg	Glu	Lys	Asp	Met	Lys	Leu	Lys	Phe	Ser	Asn	Ser	Lys	Ala	Gly	Pro
															380
Lys	Ala	Met	Lys	Lys	Thr	Asp	Val	Glu	Glu	Ser	Lys	Gly	Ala	Ser	Arg
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Ala	Leu	Ser	Lys	Phe	Ile	Asp	Pro	Ser	Leu	Thr	Trp	Lys	Asp	Ile	Glu
															415
Glu	Leu	Lys	Ser	Lys	Thr	Asn	Leu	Pro	Ile	Val	Ile	Lys	Gly	Val	Gln
															430
Arg	Thr	Glu	Asp	Val	Val	Lys	Ala	Ala	Glu	Val	Gly	Val	Ser	Gly	Val
															445
Val	Leu	Ser	Asn	His	Gly	Gly	Arg	Gln	Leu	Asp	Phe	Ser	Arg	Ala	Pro
															460
Ile	Glu	Val	Leu	Ala	Glu	Ala	Met	Pro	Val	Leu	Glu	Glu	Arg	Lys	Leu
															480
Lys	Asp	Lys	Leu	Glu	Val	Tyr	Ile	Asp	Gly	Gly	Val	Arg	Arg	Gly	Thr
															495
Asp	Ile	Leu	Lys	Ala	Leu	Cys	Leu	Gly	Ala	Lys	Gly	Val	Gly	Leu	Gly
															510
Arg	Pro	Phe	Leu	Tyr	Ala	Asn	Ser	Cys	Tyr	Gly	Arg	Asp	Gly	Val	Glu
															525
Lys	Ala	Ile	Glu	Ile	Leu	Arg	Asp	Glu	Val	Glu	Met	Ser	Met	Arg	Leu
															540
Leu	Gly	Val	Ala	Ser	Val	Ala	Glu	Leu	Lys	Pro	Glu	Leu	Leu	Asp	Leu
															560
Ser	Thr	Leu	Lys	Ala	Arg	Thr	Val	Ala	Val	Pro	Asn	Asp	Val	Leu	Tyr
															575
Asn	Glu	Val	Tyr	Glu	Gly	Pro	Thr	Leu	Thr	Asp	Phe	Glu	Asp	Ala	
															590

<210> SEQ ID NO 57
 <211> LENGTH: 4722
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Zygosaccharomyces rouxii.

<400> SEQUENCE: 57

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcocct	180
tattagcctt gatagtgtg aaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg	240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg	300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag	360
attccaaacc ctaaagtgtc cgaatttca atagggcgaa cttgaagaat aaccaaggtc	420
aataatata ctttttagtat aacctgaaa tttgcocctat agaaatctag ggtttctgtg	480
tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgcagtt gtctctatca	540
atgaaaattt cgaggaagac gataaggta agataagtag ataagagaat gatacgagat	600
aaagcacaaa ttagcagaaa gaagagtggg tgcgaacaga gtaaaccgaa tcaggaatc	660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa	720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa	780
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atagcactcg agatctcccg agtttatcat tatcaatact gccatttcaa agaatacgt	900
aataattaat agtagtgatt ttcctaactt tatttagtca aaaaattggc cttttaattc	960
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cttagtttgc aataaacaca cataaacaaa caaatctaga atgtggttta gatcgaagcc	1560
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accaagggac	tacctctacg	acaagactta	catgagttct	gatttgggta	gtttcatgcc	3300
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aaccgaagtt	atctgatgta	gaaaaggatt	agagatgcta	agagatagtg	atgatatttc	3780
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tgcacagaa	aaaaacctgc	aggaaacgaa	gataaatcat	gtcgaaagct	acataaagg	4140
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gtattgttag	cggtttgaag	caggccggcg	aagaagtaac	aaaggaacct	agaggccttt	4560
tgatgttagc	agaattgtca	tgcaagggtc	ccctagctac	tggagaatat	actaagggtta	4620
ctggtgacat	tgcgaagagc	gacaaaagatt	ttgttatcgg	ctttattgct	caaagagaca	4680
tgggtggaag	agatgaaggt	tacgattggt	tgattatgac	ac		4722

<210> SEQ ID NO 58

<211> LENGTH: 598

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<212> TYPE: PRT

<213> ORGANISM: Zygosaccharomyces rouxii

<400> SEQUENCE: 58

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Met Trp Phe Arg Ser Lys Pro Ile Trp Lys Leu Ser Glu Val Thr Ala
1          5          10          15
Arg Ser Leu Arg Gly Ile Asn Thr Lys Arg Ser Phe Thr Trp Asn Ser
20          25          30
Lys Gly Ser Asn Ala Phe Val Arg Ser Ser Ile Leu Lys Lys Arg Ser
35          40          45
Met Ala Thr Ala Leu Leu Val Thr Ala Val Ala Ala Ala Ser Ser Val
50          55          60
Lys Leu Ser Ser Arg Pro Leu Asp Asn Gly Pro Lys Val Asp Met Ser
65          70          75          80
Lys Pro Ala Ile Ser Pro Asp Glu Val Ala Lys His Asn Ser Pro Asp
85          90          95
Asp Cys Trp Val Val Ile Asp Gly Tyr Val Tyr Asp Leu Thr Glu Phe
100         105         110
Ala Pro Val His Pro Gly Gly Pro Thr Val Ile Lys Ser Asn Ala Gly
115         120         125
Lys Asp Val Ser Ala Ile Phe Asp Pro Leu His Ala Pro Asp Val Ile
130         135         140
Glu Lys Phe Ile Asp Pro Ser Lys Arg Leu Gly Pro Leu Glu Gly Asp
145         150         155         160
Met Pro Lys Glu Leu Val Cys Ala Pro Phe Thr Pro Gly Glu Thr Pro
165         170         175
Asp Asp Val Gln Arg Lys Leu Lys Leu Arg Ala Arg Leu Pro Pro Leu
180         185         190
Asp Ala Ile Thr Asn Leu Tyr Asp Phe Glu Phe Leu Ala Ser Gln Val
195         200         205
Leu Thr Lys Gln Ala Trp Ala Tyr Tyr Ser Ser Gly Ala Asp Asp Glu
210         215         220
Ile Thr Met Arg Glu Asn His Phe Ala Tyr His Arg Ile Phe Phe Lys
225         230         235         240
Pro Lys Val Leu Val Asn Val Ala Glu Val Asp Thr Lys Thr Glu Met
245         250         255
Leu Gly Ala Pro Val Asp Val Pro Phe Tyr Val Thr Ala Thr Ala Leu
260         265         270
Cys Lys Leu Gly Asn Pro Ala Glu Gly Glu Lys Asp Ile Ala Arg Gly
275         280         285
Cys Gly Ser Gly Glu Lys Lys Val Pro Gln Met Val Ser Thr Leu Ala
290         295         300
Ser Cys Ser Leu Glu Glu Val Val Asn Ala Gly Lys Glu Asp Gln Ile
305         310         315         320
Arg Trp Phe Gln Leu Tyr Met Asn Glu Asp Arg Ser Val Val Asp Gln
325         330         335
Met Ile Ser Ser Ala Glu Lys Leu Gly Tyr Lys Gly Ile Phe Val Thr
340         345         350
Val Asp Ala Pro Gly Leu Gly Asn Arg Glu Lys Asp Thr Lys Val Lys
355         360         365
Phe Ser Ser Gln Ala Gly Pro Leu Ser Val Lys Lys Lys Glu Lys Glu
370         375         380
Asp Lys Gly Lys Asp Asn Gly Glu Ser Ser Gly Ala Ser Lys Tyr Leu
385         390         395         400

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Ser Lys Phe Ile Asp Pro Ser Phe Asp Trp Asp Asp Leu Val Glu Val
405 410 415

Lys Lys Lys Thr Lys Leu Pro Ile Val Ile Lys Gly Val Gln Arg Val
420 425 430

Glu Asp Val Val Lys Ala Ala Glu Val Gly Ala Ser Gly Val Val Leu
435 440 445

Ser Asn His Gly Gly Arg Gln Leu Asp Phe Ser Arg Ser Pro Ile Glu
450 455 460

Val Leu Ala Glu Ala Gln Pro Ile Leu Lys Glu Arg Asn Phe Glu Asn
465 470 475 480

Phe Asp Val Phe Val Asp Gly Gly Ile Arg Arg Gly Thr Asp Val Val
485 490 495

Lys Ala Leu Cys Leu Gly Ala Lys Gly Val Gly Leu Gly Arg Pro Phe
500 505 510

Leu Tyr Ala Asn Ser Val Tyr Gly Lys Glu Gly Val Gln Lys Ala Ile
515 520 525

Asp Ile Leu Asn Phe Glu Val Glu Met Thr Met Arg Leu Leu Gly Val
530 535 540

Thr Ser Ile Lys Gln Leu Gly Pro Glu Leu Ile Asp Thr Ser Cys Ile
545 550 555 560

Lys Ser Arg Ser Val Gln Val Pro Arg Asp Tyr Leu Tyr Asp Lys Thr
565 570 575

Tyr Met Ser Ser Asp Leu Val Ser Phe Met Pro Pro Gln Glu Asp Glu
580 585 590

Ala Asp Gly Glu Asn Glu
595

<210> SEQ ID NO 59

<211> LENGTH: 4695

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Kluyveromyces lactis.

<400> SEQUENCE: 59

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtccct    180
tattagcctt gatagtgtg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg    300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatatat cttttagtat aaccctgaaa ttgcccctat agaaatctag ggttttctgtg    480
tggtttcceg gtgagtcata cggtcttttt gaatttcttt ttttgcagtt gtctctatca    540
atgaaaaatt cgaggaagac gataaggtta agataagtag ataagagaat gatacgagat    600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcaggggaatc    660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa    720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa    780
agtttatttc agagttcttc agacttotta actcctgtaa aaacaaaaaa aaaaaaaggc    840

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atagcactcg agatctcccg agtttatcat tatcaatact gccatttcaa agaatacgtg	900
aataattaat agtagtgatt ttcctaactt tatttagtca aaaaattggc cttttaattc	960
tgctgtaacc cgtacatgcc caaaataggg ggcgggttac acagaatata taacatcata	1020
ggtgtctggg tgaacagttt attcctggca tccactaaat ataatggagc ccgctttttt	1080
taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccaac	1140
catcagttca taggtccatt ctcttagcgc aactacacag aacaggggca caaacaggca	1200
aaaaacgggc acaacctcaa tggagtgatg caacctgctt ggagtaaatg atgacacaag	1260
gcaattgacc tacgcatgta tctatctcat tttcttacac cttctattac cttctgctct	1320
ctctgatttg gaaaaagctg aaaaaaagg ttgaaaccag ttcctgaaa ttattccctt	1380
atgtgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc	1440
ttaaacttct taaattctac ttttatagtt agtctttttt ttagtttaa acaccaagaa	1500
cttagtttcg aataaacaca cataaacaaa caaatctaga atgagatcag ccgtaagagt	1560
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cattgagggt tacgtataca atttgactga tttcatcagc gcccatcctg gtgggtccagc	1920
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aagaatcttg gttaacgtta aggaagtga cacttccact acaatgtag gtgaaaaagt	2340
cgggtttcca ttttatgtgt ctgccaccgc attgtgtaa ctaggtaacc ccaaggaagg	2400
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cggcttaggt agaccattct tatactctaa cagttgctac ggtaaagaag gtgtcaagaa	3120
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ggttgattat gacac 4695

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<210> SEQ ID NO 60

<211> LENGTH: 589

<212> TYPE: PRT

<213> ORGANISM: Kluyveromyces lactis

<400> SEQUENCE: 60

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Met Arg Ser Ala Val Arg Val Phe Gly Lys Asn Arg Val Ser Gly Leu
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Arg Phe Ala Ser Thr Ser Ser Lys Ser Pro Ile His Lys Ser Ser Ala
20           25           30
Thr Leu Arg Phe Gly Arg Ser Ala Lys Ser Gly Tyr Ser Ala Phe Ser
35           40           45
Phe Gly Lys Lys Val Ile Thr Gly Gly Ala Phe Leu Val Ala Gly Ser
50           55           60
Val Gly Ile Ala Leu Ile Ser Gln Phe Asn Asp Ser Val Glu Asn Gly
65           70           75           80
Ala Lys Val Asp Met Thr Lys Pro Lys Val Ser Pro Thr Glu Val Ala
85           90           95
Lys His Ser Ser Pro Lys Asp Cys Trp Val Val Ile Glu Gly Tyr Val
100          105          110

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Tyr Asn Leu Thr Asp Phe Ile Ser Ala His Pro Gly Gly Pro Ala Ile
 115 120 125
 Ile Glu Asn Asn Ala Gly Lys Asp Val Thr Lys Ile Phe Ala Pro Ile
 130 135 140
 His Ala Pro Asp Val Ile Glu Lys Tyr Ile Ala Pro Glu Asn Arg Ile
 145 150 155 160
 Gly Pro Leu Asp Gly Thr Met Pro Asp Asp Leu Val Cys Ala Ala Leu
 165 170 175
 Thr Pro Gly Glu Thr Pro Glu Asp Val Ala Arg Lys Glu Gln Leu Arg
 180 185 190
 Gln Ser Leu Pro Asp Ile Asp Ser Leu Val Asn Ile Tyr Asp Phe Glu
 195 200 205
 Phe Leu Ala Ser Gln Ile Leu Thr Lys Gln Ala Trp Ser Tyr Tyr Ser
 210 215 220
 Ser Ala Ala Asp Asp Glu Val Thr His Arg Glu Asn His Ala Ala Tyr
 225 230 235 240
 His Arg Ile Phe Phe Lys Pro Arg Ile Leu Val Asn Val Lys Glu Val
 245 250 255
 Asp Thr Ser Thr Thr Met Leu Gly Glu Lys Val Gly Val Pro Phe Tyr
 260 265 270
 Val Ser Ala Thr Ala Leu Cys Lys Leu Gly Asn Pro Lys Glu Gly Glu
 275 280 285
 Lys Asp Ile Ala Arg Gly Cys Gly Glu Ser Asp Val Lys Pro Val Gln
 290 295 300
 Met Ile Ser Thr Leu Ala Ser Cys Ser Leu Gln Glu Ile Val Glu Ala
 305 310 315 320
 Ala Pro Ser Lys Glu Gln Ile Gln Trp Phe Gln Leu Tyr Val Asn Ser
 325 330 335
 Asp Arg Lys Ile Thr Glu Asp Leu Ile Lys Asn Val Glu Lys Leu Gly
 340 345 350
 Leu Lys Ala Ile Phe Val Thr Val Asp Ala Pro Ser Leu Gly Asn Arg
 355 360 365
 Glu Lys Asp Ala Lys Val Lys Phe Thr Asn Ser Asn Gly Ala Lys Ala
 370 375 380
 Met Glu Lys Ser Lys Val Lys Glu Ser Lys Gly Ala Ser Arg Ala Leu
 385 390 395 400
 Ser Ser Phe Ile Asp Pro Ala Leu Asn Trp Asp Asp Ile Ile Glu Phe
 405 410 415
 Lys Lys Lys Thr Lys Leu Pro Ile Val Ile Lys Gly Val Gln Cys Val
 420 425 430
 Glu Asp Val Leu Lys Ala Ala Glu Ile Gly Val Ala Gly Val Val Leu
 435 440 445
 Ser Asn His Gly Gly Arg Gln Leu Asp Phe Ser Arg Ala Pro Ile Glu
 450 455 460
 Val Leu Ala Glu Thr Met Pro Val Leu Arg Glu Lys Lys Leu Asp Asp
 465 470 475 480
 Lys Ile Glu Ile Phe Ile Asp Gly Gly Val Arg Arg Gly Thr Asp Ile
 485 490 495
 Leu Lys Ala Leu Cys Leu Gly Ala Lys Gly Val Gly Leu Gly Arg Pro
 500 505 510
 Phe Leu Tyr Ser Asn Ser Cys Tyr Gly Lys Glu Gly Val Lys Lys Ala
 515 520 525

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Ile Glu Leu Leu Lys Asp Glu Leu Glu Met Ser Met Arg Leu Leu Gly
 530 535 540
 Val Thr Ser Ile Asp Gln Leu Ser Glu Lys Tyr Leu Asp Leu Ser Thr
 545 550 555 560
 Ile His Gly Arg Thr Val Asn Val Pro Arg Asp Asn Leu Tyr Arg Glu
 565 570 575
 Val Tyr Val Pro His Glu Pro Thr Gln Phe Leu Glu Glu
 580 585

<210> SEQ ID NO 61
 <211> LENGTH: 4704
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Kluyveromyces dobzhanskii.

<400> SEQUENCE: 61
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 attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgccct 180
 tattagcctt gatagtgtcg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg 240
 caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg 300
 gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag 360
 attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc 420
 aataatata cttttagtat aacctgaaa tttgcoctat agaaatctag ggtttctgtg 480
 tggtttccgg gtgagtcata cggtcttttt gaatttcttt tttgtagtt gtctctatca 540
 atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatcacgagat 600
 aaagcacaaa tttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcaggggaatc 660
 cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa 720
 atataaagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa 780
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 aataattaat agtagtgatt ttccctaact tatttagtca aaaaattggc cttttaattc 960
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 atttgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc 1440
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ttgggttgtc atagatggtt acgtatacaa tctaactgaa ttcatcggcg cccatcctgg	1920
tggtcacgagc ataatacaaaa acaacgcagg caaggacggt actaagatat ttgcaccaat	1980
ccatgctcca gacgttattg aaaagtacat cgctcctaaa aacagactgg gtccattgga	2040
aggtacaatg cctgaggacc gtatttgcgc tgcactcaca ccaggtgaaa ctgccgagga	2100
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agcaggcggc ggaagaagta acaaaggaac ctagaggcct tttgatgta gcagaattgt 4560
catgcaaggg ctcctagct actggagaat atactaaggg tactggtgac attgcgaaga 4620
gcgacaaaaga ttttgttatc ggttttattg ctcaaagaga catgggtgga agagatgaag 4680
gttacgattg gttgattatg acac 4704

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<210> SEQ ID NO 62

<211> LENGTH: 592

<212> TYPE: PRT

<213> ORGANISM: *Kluyveromyces dobzhanskii*

<400> SEQUENCE: 62

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Met Arg Ser Ala Val Arg Val Phe Arg Lys Asn Arg Phe Pro Thr Gly
1           5           10           15
Leu Arg Leu Ser Ser Thr Ala Ser Ser Ala Arg Gln Gly Ile Ala Leu
20          25          30
Ser Val Gly Lys Tyr Asn Lys Tyr Asn Lys Asn Gly Thr Asn Gly Ser
35          40          45
Trp Lys Ser Ser Arg Lys Ser Thr Thr Tyr Gly Gly Ala Phe Leu Val
50          55          60
Ala Ser Ala Val Ala Ile Ala Phe Ser Ser Gln Ile Thr Gln Ser Leu
65          70          75          80
Glu Asn Gly Val Lys Val Asp Met Gly Lys Pro Ser Val Ser Pro Ala
85          90          95
Glu Val Val Lys His Ser Ser Pro Asp Asp Cys Trp Val Val Ile Asp
100         105        110
Gly Tyr Val Tyr Asn Leu Thr Glu Phe Ile Gly Ala His Pro Gly Gly
115        120        125
Pro Ala Ile Ile Lys Asn Asn Ala Gly Lys Asp Val Thr Lys Ile Phe
130        135        140
Ala Pro Ile His Ala Pro Asp Val Ile Glu Lys Tyr Ile Ala Pro Lys
145        150        155        160
Asn Arg Leu Gly Pro Leu Glu Gly Thr Met Pro Glu Asp Arg Ile Cys
165        170        175
Ala Ala Leu Thr Pro Gly Glu Thr Ala Glu Asp Val Ala Arg Lys Glu
180        185        190
Gln Leu Arg Gln Leu Leu Pro Asp Val Asn Asn Leu Thr Asn Leu Tyr
195        200        205
Asp Phe Glu Phe Leu Ala Ser Gln Ile Leu Thr Lys Gln Ala Trp Ser
210        215        220
Tyr Tyr Ser Ser Ala Ala Asp Asp Glu Val Thr Gln Arg Glu Asn His
225        230        235        240
Ala Ala Tyr His Arg Ile Phe Phe Lys Pro Arg Ile Leu Val Asn Val
245        250        255

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Lys Glu Val Asp Thr Ser Thr Thr Met Leu Gly Glu Lys Val Gly Val
 260 265 270
 Pro Phe Tyr Val Ser Ala Thr Ala Leu Cys Lys Leu Gly Asn Pro Gln
 275 280
 Glu Gly Glu Lys Asp Ile Ala Arg Gly Cys Gly Glu Ser Asp Ala Lys
 290 295 300
 Pro Val Gln Met Ile Ser Thr Leu Ala Ser Cys Ser Leu Gln Glu Ile
 305 310 315
 Val Glu Ala Ala Pro Ser Lys Glu Gln Ile Gln Trp Phe Gln Leu Tyr
 325 330 335
 Val Asn Ser Asp Arg Lys Ile Thr Asp Asp Leu Val Lys Asn Ala Glu
 340 345 350
 Lys Leu Gly Leu Lys Ala Ile Phe Val Thr Val Asp Ala Pro Ser Leu
 355 360 365
 Gly Asn Arg Glu Lys Asp Ala Lys Val Lys Phe Thr Asn Ser Asn Gly
 370 375 380
 Ala Lys Ala Met Glu Lys Ser Asn Val Asp Glu Ser Lys Gly Ala Ser
 385 390 395 400
 Arg Ala Leu Ser Ser Phe Ile Asp Pro Ala Leu Asn Trp Asp Asp Ile
 405 410 415
 Val Glu Leu Lys Thr Lys Thr Lys Leu Pro Ile Val Ile Lys Gly Val
 420 425 430
 Gln Cys Val Glu Asp Val Leu Lys Ala Ala Glu Val Gly Ala Ala Gly
 435 440 445
 Val Val Leu Ser Asn His Gly Gly Arg Gln Leu Asp Phe Ser Arg Ala
 450 455 460
 Pro Ile Glu Val Leu Ala Glu Thr Met Pro Ala Leu Arg Glu Lys Lys
 465 470 475 480
 Leu Asp Asp Lys Leu Glu Val Phe Ile Asp Gly Gly Val Arg Arg Gly
 485 490 495
 Thr Asp Ile Leu Lys Ala Leu Cys Leu Gly Ala Lys Gly Val Gly Leu
 500 505 510
 Gly Arg Pro Phe Leu Tyr Ser Asn Ser Cys Tyr Gly Lys Glu Gly Val
 515 520 525
 Lys Lys Ala Ile Glu Leu Leu Lys Asp Glu Leu Glu Met Ser Met Arg
 530 535 540
 Leu Leu Gly Val Thr Ser Ile Asp Gln Leu Ser Glu Lys Tyr Leu Asp
 545 550 555 560
 Leu Ser Thr Ile His Gly Arg Thr Val Ser Val Pro Arg Asp Asn Ile
 565 570 575
 Tyr Arg Asp Val Tyr Val Ala Gln Glu Pro Ser Gln Phe Arg Glu Asp
 580 585 590

<210> SEQ ID NO 63

<211> LENGTH: 4620

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Saccharomyces kluyveri.

<400> SEQUENCE: 63

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcocct 180

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tattagcctt gatagtctg aaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg	240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg	300
gcaagtgcgt ataaatataa cctattttct tatcatcata tttacttata tctttaacag	360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc	420
aataatata cttttagtat aacctgaaa ttgcoctat agaaatctag ggtttctgtg	480
tggtttccgg gtgagtcata cggctttttt gaatttcttt ttttgagtt gtctctatca	540
atgaaaattt cgaggaagac gataaggta agataagtag ataagagaat gatacgagat	600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcagggaatc	660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa	720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa	780
agtttatttc agagttcttc agacttctta actcctgtaa aaacaaaaa aaaaaaggc	840
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aataattaat agtagtgatt ttcctaactt tatttagtca aaaaattggc cttttaattc	960
tgctgtaacc cgtacatgcc caaaataggg ggccgggttac acagaatata taacatcata	1020
ggtgtctggg tgaacagttt attcctggca tccactaaat ataattggagc ccgctttttt	1080
taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccac	1140
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atgtgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc	1440
ttaaactctt taaattctac ttttatagtt agtctttttt ttagtttaaa acaccaagaa	1500
cttagtttgc aataaacaca cataaacaaa caaatctaga atgtttaaaa gagccttttt	1560
gagacctcaa ttgtggtcct cgacgactcg tcgctgcaca atagctagca ttcggtgcta	1620
ctcgaacaaa ccgttaaaac cggcctcagc tagtaagttg actcttggtt tgatcgggtg	1680
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tgccctcaca acgagactag atgaattagc gcctccaagc tattgccagg atcctaagaa	1800
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gccccaccaa agaccacaga ttatcctatt tccacgtact acatcagatg tttccgagat	1980
tttgaagtt tgccacgaat acgaggtgcc agttatccca ttttccgggtg gtacttctct	2040
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tatgaacaaa attttgaagc tcaacaagca ggaactggat gttgagggtc aagccgggtg	2160
accatgggaa gatctaaacg attatttgaa ttctgaaggt gtcttatttg gttgcatcc	2220
tggtccaggt gctcaaatcg gtggtgtgat tgctaactcc tgttccggta ctaatgctta	2280
ccgttacggt acgatgaag aaaaacatagt gaacatcact gtggtcctag ctgatggtag	2340
tgttgtaag actaagagac gtccatgaaa atcaagtgtc ggttacaact tgaacgggtt	2400
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tccaacgttg tttttcaaga ttggaggaag atctcaaaat aacgtcaaag aattggttga 2700
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gttaggtgct gaaacagttg atctaagtac acagttaaaa ttagccttgg atccaaagag 3180
aatattgaat ccagacaaga tcttcaagat tgatcctaac gagcatgaac actgattaat 3240
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tggatatctt gactgatttt tccatggagg gcacagtaa gccgctaaag gcattatccg 4260
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acggtgtggt gggcccagggt attgtagcgt gtttgaagca ggcggcggaa gaagtaacaa 4440
aggaacctag aggccttttg atgtagcag aattgtcatg caagggtccc ctactactg 4500
gagaatatac taagggtact gttgacattg cgaagagcga caaagatttt gttatcggtc 4560
ttattgctca aagagacatg ggtggaagag atgaaggtta cgattggttg attatgacac 4620

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<210> SEQ ID NO 64

<211> LENGTH: 564

<212> TYPE: PRT

<213> ORGANISM: *Saccharomyces kluyveri*

<400> SEQUENCE: 64

Met Phe Lys Arg Ala Phe Leu Arg Pro Gln Leu Trp Ser Ser Thr Thr

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1	5	10	15
Arg Arg Cys Thr Ile Ala Ser Ile Arg Cys Tyr Ser Lys Gln Pro Leu 20 25 30			
Lys Pro Ala Ser Ala Ser Lys Leu Thr Leu Gly Leu Ile Gly Gly Ile 35 40 45			
Gly Leu Gly Ser Ala Ala Thr Ala Tyr Gly Leu Leu Met Gly Asp Asp 50 55 60			
Asp Thr Ser Ala Ser Thr Thr Arg Leu Asp Glu Leu Ala Pro Pro Ser 65 70 75 80			
Tyr Cys Gln Asp Pro Lys Lys Phe Glu Asp Ala Ile Ile Lys Leu Lys 85 90 95			
His Ile Leu Asp Asp Asn Pro Asp Asn Phe Ser Val Ser Lys Ser Glu 100 105 110			
Leu Asp Ala His Ser Asp Thr Tyr Phe Asn Ser His His Ala Arg Pro 115 120 125			
Asp Gln Arg Pro Gln Ile Ile Leu Phe Pro Arg Thr Thr Ser Asp Val 130 135 140			
Ser Glu Ile Leu Lys Val Cys His Glu Tyr Glu Val Pro Val Ile Pro 145 150 155 160			
Phe Ser Gly Gly Thr Ser Leu Glu Gly His Phe Leu Pro Thr Arg Ile 165 170 175			
Gly Cys Thr Val Val Leu Asp Met Ser Lys Tyr Met Asn Lys Ile Leu 180 185 190			
Lys Leu Asn Lys Gln Asp Leu Asp Val Glu Val Gln Ala Gly Val Pro 195 200 205			
Trp Glu Asp Leu Asn Asp Tyr Leu Asn Ser Glu Gly Val Leu Phe Gly 210 215 220			
Cys Asp Pro Gly Pro Gly Ala Gln Ile Gly Gly Cys Ile Ala Asn Ser 225 230 235 240			
Cys Ser Gly Thr Asn Ala Tyr Arg Tyr Gly Thr Met Lys Glu Asn Ile 245 250 255			
Val Asn Ile Thr Val Val Leu Ala Asp Gly Thr Val Val Lys Thr Lys 260 265 270			
Arg Arg Pro Arg Lys Ser Ser Ala Gly Tyr Asn Leu Asn Gly Leu Phe 275 280 285			
Val Gly Ser Glu Gly Thr Leu Gly Val Val Thr Gln Ala Thr Val Lys 290 295 300			
Cys His Val Met Pro Lys Ile Glu Thr Val Ala Val Val Ser Phe Pro 305 310 315 320			
Thr Val Gly Asp Ala Ala Ser Cys Ser Ser His Ile Ile Gln Glu Gly 325 330 335			
Ile Gln Leu Asn Ala Met Glu Leu Leu Asp Asp Asn Met Met Lys Val 340 345 350			
Ile Asn Gln Ser Gly Ala Thr Ser Lys Thr Asp Trp Val Glu Ser Pro 355 360 365			
Thr Leu Phe Phe Lys Ile Gly Gly Arg Ser Gln Asn Asn Val Lys Glu 370 375 380			
Leu Val Asp Glu Val Ser Lys Ile Ala Gln Lys Tyr His Cys Lys Lys 385 390 395 400			
Phe Glu Phe Ala Ser Ser Asp Asp Glu Lys Leu Glu Leu Trp Glu Ala 405 410 415			
Arg Lys Val Ala Leu Trp Ser Thr Ile Asn Ala Gly Lys Ala Lys Asp 420 425 430			

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Ser Asn Ser Asn Val Trp Thr Thr Asp Val Ala Val Pro Leu Ser Gln
 435 440 445

Phe Ala Pro Val Ile Glu Ala Thr Lys Gln Glu Met Asn Ala Ser Gly
 450 455 460

Leu Ile Thr Thr Leu Val Gly His Ala Gly Asp Gly Asn Phe His Ala
 465 470 475 480

Phe Ile Val Tyr Asn Asp Asp Gln Arg Asp Ile Ala Glu Lys Ile Val
 485 490 495

Asp Asn Met Val His Arg Ala Ile Glu Ala Glu Gly Thr Cys Thr Gly
 500 505 510

Glu His Gly Val Gly Ile Gly Lys Arg Lys Phe Leu Leu His Glu Leu
 515 520 525

Gly Ala Glu Thr Val Asp Leu Met Arg Gln Leu Lys Leu Ala Leu Asp
 530 535 540

Pro Lys Arg Ile Leu Asn Pro Asp Lys Ile Phe Lys Ile Asp Pro Asn
 545 550 555 560

Glu His Glu His

<210> SEQ ID NO 65

<211> LENGTH: 4521

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Saccharomyces bayanus.

<400> SEQUENCE: 65

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgtcctt   180
tattagcctt gatagtgtcg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg   240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg   300
gcaagtgcgt ataaattaaa cctatttctt tatcatcata tttacttata tctttaacag   360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc   420
aataatatat cttttagtat aacctgaaa tttgcctat agaaatctag ggtttctgtg   480
tggtttccgg gtgagtcata cggtttttt gaatttcttt tttgtagtt gtctctatca   540
atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat   600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcaggggaatc   660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgtatatatc tggaacagaa   720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa   780
agtttatttc agagtcttc agactctta actcctgtaa aaacaaaaaa aaaaaaggc   840
atagcactcg agatctcccg agtttatcat tatcaatact gccatttcaa agaatacgta   900
aataattaat agtagtgatt ttcttaactt tatttagtca aaaaattggc cttttaattc   960
tgctgtaacc cgtacatgcc caaaataggg ggcgggttac acagaatata taacatcata  1020
gggtgtctggg tgaacagttt attcctggca tccactaaat ataattggagc ccgctttttt  1080
taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccaac  1140
catcagttca taggtccatt ctcttagcgc aactacacag aacaggggca caaacaggca  1200
aaaaacgggc acaacctcaa tggagtgatg caacctgctt ggagtaaatg atgacacaag  1260

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atctgactaa	taagtatata	aagacggtag	gtattgattg	taattctgta	aatctatttc	1440
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gtcattttat	aatgaagact	ggatgagaaa	atataaaggc	cagtccaagt	tagttttgag	1860
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gttgatttca	tcattagcca	atctgaaaca	aataagagat	ttcgaccctg	tgtcagggat	2040
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catcatctca	tggatctgca	catgaacaaa	caccagagtc	aaacgacggt	gaaattgagg	3480
ctactgcgcc	aattgatgac	aatacagacg	atgataacaa	accgaagtta	tctgatgtag	3540
aaaaggatta	gagatgctaa	gagatagtga	tgatatttca	taaataatgt	aattctatat	3600

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acgattggtt gattatgaca c 4521
    
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<210> SEQ ID NO 66
<211> LENGTH: 530
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces bayanus
    
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<400> SEQUENCE: 66

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Met Leu Arg Asn Ile Leu Val Arg Gly Ala Gly Lys His Leu Lys Tyr
 1          5          10          15
Gly Cys Arg Tyr Val Lys Ser Ser Ala Leu Leu Gly Cys Arg Thr Lys
 20          25          30
Ile Asn Tyr Tyr Ser Thr Lys Ile Gln Thr Lys Leu Thr Ser Glu Asn
 35          40          45
Tyr Pro Asp Val Gln Arg Asp Ala Arg Phe Lys Lys Leu Thr Ser Asp
 50          55          60
Asp Leu Asn Tyr Phe Arg Ser Ile Leu Ser Glu Gln Glu Val Leu Gln
 65          70          75          80
Ala Ser Glu Leu Glu Asp Leu Ser Phe Tyr Asn Glu Asp Trp Met Arg
 85          90          95
Lys Tyr Lys Gly Gln Ser Lys Leu Val Leu Arg Pro Lys Thr Val Glu
100          105          110
Lys Ile Ser Leu Ile Leu Asn Tyr Cys Asn Asp Glu Lys Ile Ala Val
115          120          125
Val Pro Gln Gly Gly Asn Thr Gly Leu Val Gly Gly Ser Val Pro Ile
130          135          140
Phe Asp Glu Leu Ile Leu Ser Leu Ala Asn Leu Asn Lys Ile Arg Asp
145          150          155          160
Phe Asp Pro Val Ser Gly Ile Leu Lys Cys Asp Ala Gly Val Ile Leu
165          170          175
Glu Asn Ala Asn Asn Tyr Val Met Glu Gln Asn Tyr Met Phe Pro Leu
180          185          190
Asp Leu Gly Ala Lys Gly Ser Cys His Val Gly Gly Val Val Ala Thr
195          200          205
    
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Asn Ala Gly Gly Leu Arg Leu Leu Arg Tyr Gly Ser Leu His Gly Ser
 210 215 220

Val Leu Gly Leu Glu Val Val Met Pro Asn Gly Gln Ile Val Asn Ser
 225 230 235 240

Met His Ser Met Arg Lys Asp Asn Thr Gly Tyr Asp Leu Lys Gln Leu
 245 250 255

Phe Ile Gly Ser Glu Gly Thr Ile Gly Ile Ile Thr Gly Val Ser Val
 260 265 270

Leu Thr Val Pro Lys Pro Lys Ala Phe Asn Val Ser Tyr Leu Ala Val
 275 280 285

Glu Ser Phe Glu Asp Val Gln Lys Val Phe Val Arg Ala Arg Gln Glu
 290 295 300

Leu Ser Glu Ile Leu Ser Ala Phe Glu Phe Met Asp Ser Lys Ser Gln
 305 310 315 320

Ile Leu Ala Lys Ser Gln Leu Lys Asp Ala Thr Phe Pro Leu Glu Asp
 325 330 335

Glu His Pro Phe Tyr Ile Leu Ile Glu Thr Ser Gly Ser Asn Lys Asp
 340 345 350

His Asp Asp Ser Lys Leu Glu Thr Phe Leu Glu Ser Val Met Glu Glu
 355 360 365

Gly Val Val Thr Asp Gly Val Val Ala Gln Asp Glu Thr Glu Leu Gln
 370 375 380

Asn Leu Trp Lys Trp Arg Glu Met Ile Pro Glu Ala Ser Gln Ala Asn
 385 390 395 400

Gly Gly Val Tyr Lys Tyr Asp Val Ser Leu Pro Leu Lys Asp Leu Tyr
 405 410 415

Ser Leu Val Asp Ala Thr Asn Ala Arg Leu Ser Glu Ala Gly Leu Val
 420 425 430

Gly Asp Ser Pro Lys Pro Val Val Gly Ala Ile Gly Tyr Gly His Val
 435 440 445

Gly Asp Gly Asn Leu His Leu Asn Val Ala Val Arg Glu Tyr Asn Lys
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Glu Ile Glu Lys Val Leu Glu Pro Phe Val Tyr Glu Phe Val Ser Ser
 465 470 475 480

Lys His Gly Ser Val Ser Ala Glu His Gly Leu Gly Phe Gln Lys Lys
 485 490 495

Asn Tyr Ile Gly Tyr Ser Lys Ser Pro Glu Glu Val Thr Met Ile Lys
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Asp Leu Lys Ala His Tyr Asp Pro Asn Ala Ile Leu Asn Pro Tyr Lys
 515 520 525

Tyr Val
 530

<210> SEQ ID NO 67
 <211> LENGTH: 4721
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Aspergillus fumigatus.

<400> SEQUENCE: 67

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcocct 180

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caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg	300
gcaagtgcgt ataaattaaa cctattttct tatcatcata tttacttata tctttaacag	360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc	420
aataatata cttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg	480
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<210> SEQ ID NO 68

<211> LENGTH: 597

<212> TYPE: PRT

<213> ORGANISM: Aspergillus fumigatus

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<400> SEQUENCE: 68

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 Ala Arg Ile Ser Ala Asn Asp Lys Pro Gln Ser Phe Lys Asn Gln Leu
 35 40 45
 Tyr Glu Ser Thr Gln Gln Arg Leu Arg Arg Glu Arg Ala Glu Gln Glu
 50 55 60
 Arg Phe Ser Gln Tyr Gln Thr Gln Ser Pro Gly Gly Arg Tyr Ala Ala
 65 70 75 80
 Leu Thr Phe Ala Leu Val Phe Phe Ser Thr Gly Ala Tyr Tyr Leu Gly
 85 90 95
 Ser Leu Lys Pro Ala Ser Leu Pro Thr Ser Ser Thr Thr Thr Leu Phe
 100 105 110
 Glu Ile Glu Pro Pro Leu His Asn Ile Ser Pro Ala Asn Leu Gln Ala
 115 120 125
 Ala Trp Ala Asp Phe Val Glu Ile Leu Gly Lys Glu Asn Val Ser Thr
 130 135 140
 Glu His Gly Asp Leu Asp Val His Ser Gly Ser Asp Trp Ser Ser Tyr
 145 150 155 160
 Thr Leu Lys Lys Asp Glu Arg Pro Phe Leu Val Leu Tyr Pro Ser Thr
 165 170 175
 Thr Glu Glu Val Ser Arg Ile Met Lys Val Cys His Gln Arg Val Ile
 180 185 190
 Pro Val Thr Pro Tyr Ser Gly Gly Thr Ser Leu Glu Gly His Phe Ala
 195 200 205
 Pro Thr Arg Gly Gly Val Cys Ile Asp Phe Arg Arg Met Asn Arg Ile
 210 215 220
 Leu Glu Leu His Lys Lys Asp Leu Asp Val Val Val Gln Pro Ala Val
 225 230 235 240
 Gly Trp Glu Asp Leu Asn Glu Glu Leu Ser Lys Asp Gly Leu Phe Phe
 245 250 255
 Pro Pro Asp Pro Gly Pro Gly Ala Met Ile Gly Gly Met Val Gly Thr
 260 265 270
 Gly Cys Ser Gly Thr Asn Ala Tyr Arg Tyr Gly Thr Met Arg Glu Trp
 275 280 285
 Val Leu Ser Leu Thr Val Val Leu Ala Asp Gly Thr Val Ile Lys Thr
 290 295 300
 Arg Gln Arg Pro Arg Lys Ser Ser Ala Gly Tyr Asp Leu Thr Arg Leu
 305 310 315 320
 Phe Ile Gly Ser Glu Gly Thr Leu Gly Leu Val Thr Glu Ala Thr Leu
 325 330 335
 Lys Leu Thr Val Lys Pro Lys Ser Gln Ser Val Ala Val Ala Ser Phe
 340 345 350
 Pro Ser Ile His Asn Ala Ala Glu Cys Val Thr Arg Val Val Glu Glu
 355 360 365
 Gly Ile Pro Val Ala Gly Val Glu Ile Leu Asp Asp Val Gln Met Lys
 370 375 380
 Cys Ile Asn Asp Ser Arg Thr Thr Arg Arg Gln Trp Lys Glu Ser Pro
 385 390 395 400
 Thr Leu Phe Phe Lys Phe Thr Gly Thr Pro Val Gly Val Lys Glu Gln

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Ile	Glu	Leu	Val	Arg	Lys	Ile	Val	Ser	Ser	Ser	Ala	Gly	Gln	Ser	Phe
	420							425					430		
Glu	Phe	Ala	Arg	Gly	Glu	Asp	Glu	Met	Lys	Glu	Leu	Trp	Ser	Ala	Arg
	435					440						445			
Lys	Glu	Ala	Leu	Trp	Ser	Val	Met	Ser	Met	Arg	Arg	Gly	Pro	Glu	Asp
	450					455						460			
Arg	Val	Trp	Thr	Thr	Asp	Val	Ala	Val	Pro	Met	Ser	Lys	Leu	Pro	Asp
	465				470					475					480
Ile	Ile	Glu	Ala	Thr	Lys	Gln	Asp	Met	Thr	Glu	Ser	Gly	Leu	Leu	Ala
				485					490						495
Gly	Ile	Cys	Gly	His	Val	Gly	Asp	Gly	Asn	Phe	His	Ala	Ile	Ile	Leu
			500					505							510
Phe	Asn	Glu	Asp	Glu	Lys	Lys	Ile	Ala	Glu	Gly	Val	Val	His	Arg	Met
		515					520						525		
Val	Lys	Arg	Ala	Val	Glu	Met	Glu	Gly	Thr	Val	Thr	Gly	Glu	His	Gly
		530				535						540			
Val	Gly	Leu	Ile	Lys	Arg	Asp	Tyr	Leu	Gln	His	Glu	Val	Gly	Glu	Thr
		545			550					555					560
Thr	Val	Asp	Thr	Met	Arg	Arg	Ile	Lys	Met	Ala	Leu	Asp	Pro	Leu	Arg
			565						570						575
Leu	Leu	Asn	Cys	Asp	Lys	Val	Val	Arg	Val	Glu	Gln	Pro	Thr	Thr	Val
			580					585							590
Glu	Leu	Lys	Lys	Trp											
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<210> SEQ ID NO 69
 <211> LENGTH: 4652
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Kluyveromyces lactis.

<400> SEQUENCE: 69

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attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcctct    180
tattagcctt gatagtgtcg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg    240
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gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag    360
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aatagcagaa tgggcagaca ttacgaaatgc acacggtgtg gtgggccag gtattgttag 4440
cggtttgaag caggcggcgg aagaagtaac aaaggaacct agaggccttt tgatgttagc 4500
agaattgtca tgcaagggct ccttagctac tggagaatat actaagggta ctgttgacat 4560
tgcaagagc gacaaagatt ttgttatcgg ctttattgct caaagagaca tgggtggaag 4620
agatgaaggt tacgattggt tgattatgac ac 4652

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<210> SEQ ID NO 70

<211> LENGTH: 576

<212> TYPE: PRT

<213> ORGANISM: *Kluyveromyces lactis*

<400> SEQUENCE: 70

```

Met Phe Arg Phe Val Gly Arg Ser Gly Phe Ala Leu Arg Gly Ser Leu
 1           5           10          15
Gln Leu Arg Lys Asp Val Leu Arg Ser Arg Thr Thr Ala Val Ala Lys
 20          25          30
Arg His Tyr Ser Ser Ser Asn Gly Asn Asn Gly Gly Gly Phe Ser Ser
 35          40          45
Ala Ile Leu Ser Val Leu Gly Gly Ser Leu Ile Gly Gly Gly Phe Val
 50          55          60
Ala Tyr Ala Leu Gly Ser Gln Phe Glu Lys Glu Lys Ser Val Ser Asp
 65          70          75          80
Leu Ser Ile Ala Arg Leu Glu Asp Leu Asp Ser Pro Glu Tyr Cys Asp
 85          90          95
Lys Glu Thr Phe Ala Lys Ala Leu Val Glu Leu Lys Asp Val Leu Glu
100         105         110
Asn Asp Pro Glu Asn Phe Thr Val Ala Lys Asp Asp Leu Asp Ala His
115         120         125

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Ser Asp Thr Tyr Phe Asn Ser His His Ala Glu Ala Asn Gln Arg Pro
 130 135 140
 Glu Ile Val Leu Tyr Pro Arg Asn Thr Glu Asp Val Ser Lys Leu Leu
 145 150 155 160
 Lys Ile Cys His Lys Tyr Ser Ile Pro Val Ile Pro Phe Ser Gly Gly
 165 170 175
 Thr Ser Leu Glu Gly His Phe Leu Pro Thr Arg Pro Gly Ser Cys Val
 180 185 190
 Val Leu Asp Ile Ser Lys Tyr Leu Asn Lys Ile Ile Gln Leu Asn Lys
 195 200 205
 Glu Asp Leu Asp Val Val Val Gln Gly Gly Val Pro Trp Glu Glu Leu
 210 215 220
 Asn Glu Tyr Leu Asn Asp His Gly Leu Leu Phe Gly Cys Asp Pro Gly
 225 230 235 240
 Pro Gly Ala Gln Ile Ala Gly Cys Ile Ala Asn Ser Cys Ser Gly Thr
 245 250 255
 Asn Ala Tyr Arg Tyr Gly Thr Met Lys Glu Asn Val Val Asn Ile Thr
 260 265 270
 Met Cys Met Ala Asp Gly Thr Ile Val Lys Thr Lys Arg Arg Pro Arg
 275 280 285
 Lys Ser Ser Ala Gly Tyr Asn Leu Asn Gly Leu Ile Ile Gly Ser Glu
 290 295 300
 Gly Thr Leu Gly Ile Val Thr Glu Ala Thr Ile Lys Cys His Val Arg
 305 310 315 320
 Ser Thr Phe Glu Thr Val Ala Val Val Pro Phe Pro Thr Val Ser Asp
 325 330 335
 Ala Ala Ser Cys Ser Ser His Leu Ile Gln Ala Gly Ile Gln Leu Asn
 340 345 350
 Ala Met Glu Leu Leu Asp Asp Asn Met Met Lys Ile Ile Asn Gln Ser
 355 360 365
 Gly Ala Thr Ser Lys Asp Asn Trp Val Glu Ser Pro Thr Leu Phe Phe
 370 375 380
 Lys Ile Gly Gly Arg Ser Glu Gln Ile Ile Gln Glu Val Ile Lys Glu
 385 390 395 400
 Val Glu Lys Ile Ala Ser Gln His Asn Asn Thr Lys Phe Glu Phe Ala
 405 410 415
 Thr Asp Glu Asp Ser Lys Leu Glu Leu Trp Glu Ala Arg Lys Val Ala
 420 425 430
 Leu Trp Ser Thr Ile Asp Thr Gly Arg Lys Thr Asn Pro Asp Ala Asn
 435 440 445
 Ile Trp Thr Thr Asp Val Ala Val Pro Ile Ser Lys Phe Ala Asp Val
 450 455 460
 Ile Asn Ala Thr Lys Glu Glu Met Asn Ala Ser Gly Leu Leu Thr Ser
 465 470 475 480
 Leu Val Gly His Ala Gly Asp Gly Asn Phe His Ala Phe Ile Ile Tyr
 485 490 495
 Asn Thr Glu Gln Arg Lys Thr Ala Glu Thr Ile Val Glu Asn Met Val
 500 505 510
 Lys Arg Ala Ile Asp Ala Glu Gly Thr Cys Thr Gly Glu His Gly Val
 515 520 525
 Gly Ile Gly Lys Arg Asp Tyr Leu Leu Glu Glu Val Gly Glu Asp Thr
 530 535 540
 Val Ala Val Met Arg Lys Leu Lys Leu Ala Leu Asp Pro Lys Arg Ile

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545	550	555	560	
Leu	Asn	Pro	Asp	Lys
	Ile	Phe	Lys	Ile
	Asp	Pro	Asn	Asp
	His	Gln	His	
	565	570	575	

<210> SEQ ID NO 71
 <211> LENGTH: 4676
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and *Kluyveromyces dobzhanskii*.

<400> SEQUENCE: 71

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gtatttccga gatacgatta ctccagttcc tcttacaaga aatgcataaa aatagttaca    120
attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcacct    180
tattagcctt gatagtgtcg aaaaaaagaa aaaaaacaaa aaaaagaaat aaaataacgg    240
caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg    300
gcaagtgcgt ataaatataa cctatttctt tatcatcata tttacttata tctttaacag    360
attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc    420
aataatata cttttagtat aaccctgaaa ttgcccctat agaaatctag ggtttctgtg    480
tggtttccgg gtgagtcata cggccttttt gaatttcttt ttttgcagtt gtctctatca    540
atgaaaattt cgaggaagac gataagggtta agataagtag ataagagaat gatacgagat    600
aaagcacaaa ttagcagaaa gaagagtggg tgccaacaga gtaaaccgaa tcagggaatc    660
cctttttgca aaaacatcaa ttatcctttt ctttttttta cgatatatc tggaacagaa    720
atatataagt tactattata cttatagttg gatccagttt ttaatctgtc gtcaatcgaa    780
agtttatttc agagtcttcc agactcttta actcctgtaa aaacaaaaaa aaaaaaaggc    840
atagcactcg agatctcccg agtttatcat tatcaatact gccatttcaa agaatacgtc    900
aataattaat agtagtgatt ttcctaactt tatttagtca aaaaattggc cttttaattc    960
tgctgtaacc cgtacatgcc caaaataggg ggccgggttac acagaatata taacatcata    1020
gggtgtctgg tgaacagttt attcctggca tccactaaat ataattggagc ccgctttttt    1080
taagctggca tccagaaaaa aaaagaatcc cagcaccaaa atattgtttt cttcaccaac    1140
catcagttca taggtccatt ctcttagcgc aactacacag aacaggggca caaacaggca    1200
aaaaacgggc acaacctcaa tggagtgatg caacctgctt ggagtaaatg atgacacaag    1260
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atctgactaa taagtatata aagacggtag gtattgattg taattctgta aatctatttc    1440
ttaaacttct taaattctac ttttatagtt agtctttttt ttagtttaaa acaccaagaa    1500
cttagtttgc aataaacaca cataaacaaa caaatctaga atgttcaaat ttgttggtag    1560
aagtggcaca gccttgagat cttcattgca agcacgtaga aatggagcgg tttggaagag    1620
aggatacgc tctggttctg gtacggtag tggttcaggt agtggctcat catcaactcgt    1680
tctaggagtg ttagccggat ctatgatagg aggtggatt gtggcataca cattaggtaa    1740
ccaattcgaa aagacaaaa aggtttccgg ctctctgct gctgccgag cttctgattt    1800
atccatcttg aagtggagg acttagagag cccagtttat tgtgactccg aaactttttc    1860
aacagcagtt gaagagtaa agaccgtgct gaacaatgac cctgaaaact ttaccacagc    1920

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taaagcagat	cttgacagcc	attctgatac	ttacttcaac	tctcatcacg	ctgaaccaga	1980
tcaaagacca	gaaatcgttt	tgttcocctag	aaacactgaa	gatgtctctc	agttgttgaa	2040
gatctgtcat	aagtactcta	tcocctgtcat	tccatthttct	ggcggtagat	cattggaag	2100
tcatttecta	ccaactagac	ctggttocctg	cgtegtacta	gatattagta	aatacttgaa	2160
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cgggactatg	aaagagaatg	tagttaacat	cacaatgtgt	atggccgatg	gtaccattat	2400
caagacaaa	aagcgtccac	gtaaatctgc	agccggttac	aatcttaatg	gtctaattgt	2460
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atcacatctt	atacaagcgg	gtattcaact	aaacgccatg	gaactgttag	atgataacat	2640
gatgaagatt	atcaatcaat	ctggggcaac	ttccaaaatt	gattgggttg	agtcaccaac	2700
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cctcccacat	ccgctctaac	cgaaaaggaa	ggagttagac	aacctgaagt	ctaggtccct	3420
atthattttt	ttatagttat	gtagtatta	agaacgttat	ttatatttca	aatthttctt	3480
ttthttctgt	acaaacgcgt	gtacgcatgt	aacgggcaga	cggccggcca	taacttcgta	3540
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gacttagatt	ggatatata	cgcatatgtg	gtgttgaaga	aacatgaaat	tgcccagtat	4020
tcttaaccca	actgcacaga	acaaaaacct	gcaggaaacg	aagataaatc	atgtcgaaag	4080
ctacataata	ggaacgtgct	gctactcctc	ctagtcctgt	tgctgccaag	ctatthtaata	4140
tcatgcacga	aaagcaaaaca	aacttgtgtg	cttcattgga	tgthctgacc	accaaggaat	4200
tactggagtt	agttgaagca	ttaggtocca	aaatthgttt	actaaaaaca	catgtggata	4260

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tcttgactga tttttccatg gagggcacag ttaagccgct aaaggcatta tccgccaagt 4320
acaatttttt actcttcgaa gacagaaaat ttgctgacat tggtaataca gtcaaattgc 4380
agtactctgc gggtgtatac agaatagcag aatgggcaga cattacgaat gcacacggtg 4440
tggtggggccc aggtattggt agcggtttga agcaggcggc ggaagaagta acaaaggaac 4500
ctagaggcct tttgatgtta gcagaattgt catgcaaggg ctcctagct actggagaat 4560
atactaaggg tactgttgac attgcgaaga gcgacaaaga ttttgttatc ggctttattg 4620
ctcaaagaga catgggtgga agagatgaag gttacgattg gttgattatg acacgc 4676

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<210> SEQ ID NO 72

<211> LENGTH: 582

<212> TYPE: PRT

<213> ORGANISM: Kluyveromyces dobzhanskii

<400> SEQUENCE: 72

```

Met Phe Lys Phe Val Gly Arg Ser Gly Thr Ala Leu Arg Ser Ser Leu
 1           5           10           15
Gln Ala Arg Arg Asn Gly Ala Val Trp Lys Arg Gly Tyr Ala Ser Gly
          20           25           30
Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Ser Ser Leu Val Leu
          35           40           45
Gly Val Leu Ala Gly Ser Met Ile Gly Gly Gly Ile Val Ala Tyr Thr
          50           55           60
Leu Gly Asn Gln Phe Glu Lys Asp Lys Lys Val Ser Gly Ser Ser Ala
65           70           75           80
Ala Ala Ala Ala Ser Asp Leu Ser Ile Leu Lys Leu Glu Asp Leu Glu
          85           90           95
Ser Pro Val Tyr Cys Asp Ser Glu Thr Phe Ser Thr Ala Val Glu Glu
          100          105          110
Leu Lys Thr Val Leu Asn Asn Asp Pro Glu Asn Phe Thr Thr Ala Lys
          115          120          125
Ala Asp Leu Asp Ser His Ser Asp Thr Tyr Phe Asn Ser His His Ala
          130          135          140
Glu Pro Asp Gln Arg Pro Glu Ile Val Leu Phe Pro Arg Asn Thr Glu
145          150          155          160
Asp Val Ser Gln Leu Leu Lys Ile Cys His Lys Tyr Ser Ile Pro Val
          165          170          175
Ile Pro Phe Ser Gly Gly Thr Ser Leu Glu Gly His Phe Leu Pro Thr
          180          185          190
Arg Pro Gly Ser Cys Val Val Leu Asp Ile Ser Lys Tyr Leu Asn Lys
          195          200          205
Ile Ile His Leu Asn Lys Glu Asp Leu Asp Val Val Val Gln Gly Gly
          210          215          220
Val Pro Trp Glu Asp Leu Asn Asp Tyr Leu Ile Asp Gln Gly Leu Leu
225          230          235          240
Phe Gly Cys Asp Pro Gly Pro Gly Ala Gln Ile Ala Gly Cys Ile Ala
          245          250          255
Asn Ser Cys Ser Gly Thr Asn Ala Tyr Arg Tyr Gly Thr Met Lys Glu
          260          265          270
Asn Val Val Asn Ile Thr Met Cys Met Ala Asp Gly Thr Ile Ile Lys
          275          280          285
Thr Lys Lys Arg Pro Arg Lys Ser Ala Ala Gly Tyr Asn Leu Asn Gly
290          295          300

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Leu Ile Val Gly Ser Glu Gly Thr Leu Gly Ile Val Thr Glu Ala Thr
 305 310 315 320

Ile Lys Cys His Val Arg Ser Leu Phe Glu Thr Val Ala Val Val Pro
 325 330 335

Phe Pro Thr Val Ser Asp Ala Ala Ser Cys Ser Ser His Leu Ile Gln
 340 345 350

Ala Gly Ile Gln Leu Asn Ala Met Glu Leu Leu Asp Asp Asn Met Met
 355 360 365

Lys Ile Ile Asn Gln Ser Gly Ala Thr Ser Lys Ile Asp Trp Val Glu
 370 375 380

Ser Pro Thr Leu Phe Phe Lys Ile Gly Gly Arg Thr Glu Lys Ser Ile
 385 390 395 400

Gln Asp Val Val Asp Glu Val Glu Lys Ile Ala Ser Gln His Asn Asn
 405 410 415

Tyr Lys Phe Glu Phe Ala Thr Asp Ala Asp Ser Lys Leu Glu Leu Trp
 420 425 430

Glu Ala Arg Lys Val Ala Leu Trp Ser Thr Ile Asp Ala Gly Lys Lys
 435 440 445

Leu Asp Pro Lys Val Asn Val Trp Thr Thr Asp Val Ala Val Pro Ile
 450 455 460

Ser Arg Phe Ala Glu Val Ile Asn Ala Thr Lys Gln Glu Met Asn Ser
 465 470 475 480

Ser Gly Leu Leu Thr Ser Leu Val Gly His Ala Gly Asp Gly Asn Phe
 485 490 495

His Ala Phe Ile Ile Tyr Asn Ser Glu Gln Arg Lys Gln Ala Glu Thr
 500 505 510

Ile Val Glu Asn Met Val Lys Arg Ala Ile Asp Ala Glu Gly Thr Cys
 515 520 525

Thr Gly Glu His Gly Val Gly Ile Gly Lys Arg Asp Tyr Leu Val Glu
 530 535 540

Glu Leu Gly Glu Asp Ala Ile Ala Val Met Arg Lys Leu Lys Leu Ala
 545 550 555 560

Leu Asp Pro Lys Arg Ile Leu Asn Pro Asp Lys Ile Phe Lys Ile Asp
 565 570 575

Pro Asn Asp Asn Gln His
 580

<210> SEQ ID NO 73
 <211> LENGTH: 4704
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Kluyveromyces marxianus.

<400> SEQUENCE: 73

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gtatttcgga gatacgatta ctccagttcc tttacaaga aatgcataaa aatagttaca 120

attaattaga acaagaactt atttagaaca cgttcacact gagtaagaac tcttgcocct 180

tattagcctt gatagtgtg aaaaaaagaa aaaaaacaaa aaaaagaat aaaataacgg 240

caaacagcaa aggccacaga accgtattca tgttacttct gcaatatcaa tcacttactg 300

gcaagtgcgt ataaatataa cctattttct tatcatcata tttacttata tctttaacag 360

attccaaacc ctaaagtgtc cgaattttca atagggcgaa cttgaagaat aaccaaggtc 420

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aataatata	cttttagtat	aacctgaaa	tttgcctat	agaaatctag	ggtttctgtg	480
tggtttccgg	gtgagtcata	cggtcttttt	gaatttcttt	ttttgcagtt	gtctctatca	540
atgaaaattt	cgaggaagac	gataagggtta	agataagtag	ataagagaat	gatcacgagat	600
aaagcacaaa	ttagcagaaa	gaagagtgg	tgccaacaga	gtaaaccgaa	tcagggaaatc	660
cctttttgca	aaaacatcaa	ttatcctttt	ctttttttta	cgatatatc	tggaacagaa	720
atatataagt	tactattata	cttatagttg	gatccagttt	ttaatctgtc	gtcaatcgaa	780
agtttatttc	agagttcttc	agacttctta	actcctgtaa	aaacaaaaaa	aaaaaaaggc	840
atagcactcg	agatctcccg	agtttatcat	tatcaatact	gccatttcaa	agaatacgt	900
aataattaat	agtagtgatt	ttcctaactt	tatttagtca	aaaaattggc	cttttaattc	960
tgctgtaacc	cgtacatgcc	caaaatagg	ggcgggttac	acagaatata	taacatcata	1020
ggtgtctggg	tgaacagttt	attcctggca	tccactaaat	ataatggagc	ccgctttttt	1080
taagctggca	tccagaaaaa	aaaagaatcc	cagcaccaaa	atattgtttt	cttcaccaac	1140
catcagttca	taggtccatt	ctcttagcgc	aactacacag	aacaggggca	caaacaggca	1200
aaaaacgggc	acaacctcaa	tggagtgatg	caacctgctt	ggagtaaag	atgacacaag	1260
gcaattgacc	tacgcatgta	tctatctcat	ttctttacac	cttctattac	cttctgtct	1320
ctctgatttg	gaaaaagctg	aaaaaaaagg	ttgaaaccag	ttccctgaaa	ttattccct	1380
atgtgactaa	taagtatata	aagacggtag	gtattgattg	taattctgta	aatctatttc	1440
ttaaacttct	taaattctac	ttttatagtt	agtctttttt	ttagtttaaa	acaccaagaa	1500
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acaactcttc	tcacaacaag	tttctgacct	ctcaatagcg	agacttgaag	atttagactc	1860
ccctaagtac	tgcgacaaaa	agaccttgc	aactgctgtc	gaggaactga	agcaagtctt	1920
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cccattcttc	ggtggtagct	cacttgaagg	tcatttcatg	ccaaccagac	cagggtcatg	2160
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agaagcgaca	atcaagtgcc	atgtcagatc	aaatttcgaa	actgtcgcag	tcgttccatt	2580
cccttcagtt	gctgatgctg	cttctgttgc	ttctcatttg	atcaggctg	gcattcagtt	2640
gaacgccatg	gaattgctcg	atgataacat	gatgaagatc	atcaataagt	ccgggcaaac	2700
tagtagaaca	aattgggtag	aatcaccaac	ccttttcttc	aagatagggtg	gtagaagcga	2760
aaaagctatt	aaggaagtgt	ttaaagaggt	tgagaaaatt	gcttcacagc	acaataactc	2820

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aacagatggt gcagtcocaa taagtaaat cgcacaagtc atcaatgata caaaggagga 3000
aatgaaecg cctggcctgt taacctcatt ggtcgggtcat gctgggtgat gtaactttca 3060
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agcagaagga agaacgaagg aaggagcaca gacttagatt ggtatatata cgcataatgtg 4020
gtgttgaaga aacatgaaat tgcccagtat tcttaaccca actgcacaga acaaaaacct 4080
gcaggaacg aagataaatc atgtcgaaag ctacatataa ggaacgtgct gctactcacc 4140
ctagtcctgt tgctgccaag ctatttaata tcatgcacga aaagcaaca aacttggtgtg 4200
cttcattgga tgttcgtacc accaaggaat tactggagtt agttgaagca ttaggtccca 4260
aaatttgttt actaaaaaca catgtggata tcttgactga tttttccatg gagggcacag 4320
ttaagccgct aaaggcatta tccgccaagt acaatttttt actcttcgaa gacagaaaat 4380
ttgctgacat tggtaataca gtcaaattgc agtactctgc ggggtgtatac agaatagcag 4440
aatgggcaga cattacgaat gcacacggtg tgggtggccc aggtattggt agcggtttga 4500
agcagggcggc ggaagaagta acaaaggaac ctagaggcct tttgatgta gcagaattgt 4560
catgcaaggg ctccttagct actggagaat atactaaggg tactggtgac attgcgaaga 4620
gcgacaaaga ttttgattatc ggttttattg ctcaaagaga catgggtgga agagatgaag 4680
gttacgattg gttgattatg acac 4704

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<210> SEQ ID NO 74

<211> LENGTH: 592

<212> TYPE: PRT

<213> ORGANISM: Kluyveromyces marxianus

<400> SEQUENCE: 74

```

Met Leu Pro Arg Phe Val Val Arg Ser Gly Ala Ala Gly Arg Asn Leu
1           5           10          15

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Gly Phe Ser Phe Thr Arg Lys Cys Asp His Thr Phe Leu Ser Lys Thr
20          25          30

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-continued

450					455					460					
Val	Trp	Thr	Thr	Asp	Val	Ala	Val	Pro	Ile	Ser	Lys	Phe	Ala	Gln	Val
465					470					475					480
Ile	Asn	Asp	Thr	Lys	Glu	Glu	Met	Asn	Ala	Ser	Gly	Leu	Leu	Thr	Ser
				485					490					495	
Leu	Val	Gly	His	Ala	Gly	Asp	Gly	Asn	Phe	His	Ala	Phe	Ile	Ile	Tyr
			500					505					510		
Asn	Ala	Glu	Gln	Arg	Lys	Thr	Ala	Glu	Thr	Ile	Val	Glu	Asn	Met	Val
		515					520					525			
Lys	Arg	Ala	Ile	Asp	Ala	Glu	Gly	Thr	Cys	Thr	Gly	Glu	His	Gly	Val
530					535					540					
Gly	Ile	Gly	Lys	Arg	Glu	Phe	Leu	Val	Glu	Glu	Leu	Gly	Glu	Asp	Thr
545				550					555					560	
Ile	Ala	Val	Met	Arg	Lys	Leu	Lys	Leu	Ala	Leu	Asp	Pro	Lys	Arg	Ile
				565					570					575	
Leu	Asn	Pro	Asp	Lys	Val	Phe	Lys	Ile	Asp	Pro	Asn	Asp	His	Gln	His
			580					585					590		

<210> SEQ ID NO 75

<211> LENGTH: 4953

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Kluyveromyces dobzhanskii.

<400> SEQUENCE: 75

```

tccatggagg gcacagttaa gccgctaaag gcattatccg ccaagtacaa ttttttactc   60
ttcgaagaca gaaaatttgc tgacattggt aatacagtca aattgcagta ctctgcgggt   120
gtatacagaa tagcagaatg gccagacatt acgaatgcac acggtgtggt gggcccaggt   180
attgttagcg gtttgaagca ggcggcggaa gaagtaacaa aggaacctag aggccctttg   240
atggttagcag aattgtcatg caagggtccc ctagctactg gagaatatac taagggtact   300
gttgacattg cgaagagcga caaagatttt gttatcggct ttattgctca aagagacatg   360
gggtgaagag atgaaggtta cgattgggtg attatgacac ccggtgtggg tttagatgac   420
aagggagacg cattgggtca acagtataga accgtggatg atgtggtctc tacaggatct   480
gacattatta ttgttgaag aggactatnt gcaaaggaa gggatgctaa ggtagagggt   540
gaacgttaca gaaaagcagg ctgggaagca tatttgagaa gatgcccga gcaaaactaa   600
aaaactgtat tataagtaaa tgcattgata ctaaaactcac aaattagagc ttcaatttaa   660
ttatatcagt tattaccocg gaatctcggc cgtaagtatt tttataatga cgaaaaaaaa   720
aaaattggaa agaaaaagct tcatggcctt tataaaaaag aaccatccaa tacctcgcca   780
gaaccaagta acagtatttt acggggcaca aatcaagaac aataagacag gactgtaaaag   840
atggacgcat tgaactccaa agaacaacaa gagttccaaa aagtagtggg acaaaagcaa   900
atgaaggatt tcatgcggtt gataacttcg tataatgtat gctatacгаа gttatctcga   960
gatctcccct aaaccgtgga atatttcgga tatccttttg ttgtttccgg gtgtacaata  1020
tggacttctc cttttctggc aaccaaacc atacatcggg attcctataa taccttcggt  1080
ggctctcccta acatgtaggt ggcggagggg agatatacaa tagaacagat accagacaag  1140
acataatggg ctaacaaga ctacaccatt tacactgcct cattgatggt ggtacataac  1200
gaactaatac tgtagcccta gacttgatag ccatcatcat atcgaagttt cactaccctt  1260

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tttccatttg ccatctattg aagtaataat aggcgcgatgc aacttctttt tttttttttt	1320
ttttctctct cccccgttgt tgtctcacca tatccgcaat gacaaaaaaaa tgatggaaga	1380
cactaaagga aaaaattaac gacaaagaca gcaccaacag atgtcgttgt tccagagctg	1440
atgaggggta tctcgaagca cacgaaactt tttccttctc tcattcacgc aactactctc	1500
ctaattgagca acggtatacgc gccttccttc cagttacttg aatttgaat aaaaaaagt	1560
ttgctgtctt gctatcaagt ataatagac ctgcaattat taatcttttg tttcctcgtc	1620
attgttctcg ttccctttct tccttgtttc tttttctgca caatatttca agctatacca	1680
agcatacaat caactatctc atatacatct agaattgcata caatttctcc agaattctgac	1740
ttccctgaaat catctaagag taacactaac tcacatgaat acatgagtgcc aacaaccgat	1800
tacaataacg ggggtgctgc tgcagcccca ctttctgcta agagaaaaga gacaggaaac	1860
agctacgaac tccaagacca tgctgcgtcc gccagcgcca gtgcatctgc ttcagcctca	1920
gcatcagctt ctgcaagcgc actagctttt gattacgac cagaagatga cgatgtttca	1980
atcatgatgc aagatgaaga ggttctctgat ttgacctgga agcatgtatc taactatgca	2040
atcactagat ttacttcatt agcccatatt catccagtct caatgcaaaa catcaatcca	2100
ttccagagtg tgagaaagat gacattacac aactggaatt acttctttat gggctacggt	2160
gcgtggcttt gcgctagtgt ggctttcttt gctgtatcag tctcaacagc cccattagca	2220
gaactatacgc acaaacagac taaagatatt tcttgggggtt tgctccttagt cttgttctgt	2280
agatctgcgc gtgctatcat tttcgggtatc tggactgaca actattccag aaagtggcca	2340
tacattacat gtcttggtatt gtttttgatt tgtcagctgt gcacaccatt tgcgacaaat	2400
tacactcaat tcttaggggt gagatggatc tctggtgtgg ctatgggagg aatttacgca	2460
tgtgcaagtg caacagctat cgaggatgct ccagttaagg caagatcttt cttatccggt	2520
ctattcttta ccgcatacgc aatgggggtc attttcgcca ctgttttcta cagagcttc	2580
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aaggctagac aactgatgct tgatgatgct attgcggcaa atggcgggca acctctacct	2760
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gaaataacag gtcgtagact aggattgttg atagccttgg tcatggcagg ctgttttact	3060
tatccagcat tcatgttgaa cactggctct gccgttctcg tgggtggttt cttcctgtgg	3120
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gaagcaagag ctttagtatac tggattagct taccaacttg gaaatctcgc gtcagctgcc	3240
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cacttgaaag agtacacaga tagagttgat agagccgaag aggctgcagg catgggttagc	3480
tctgccgcat ctgtttcacc aaaaccagaa caaaacttgc ttgagaaggt gtgattaatt	3540
aatttaccag cttactatcc ttcttgaaaa tatgcactct atatctttta gttcttaatt	3600
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agaatatagc acgagccgcg gagttcattt cgttactttt gatatcgctc acaactattg 3840
cgaagcgctt cagtgaaaaa atcataagga aaagttgtaa atattattgg tagtattcgt 3900
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tatttttatac gatgattcac cacaacaatc ttgctcccgga aaagaaagca gacggagtag 4200
aagcatttga aactccttca gaccttcaag tatatatata tatatatata tatgtatatg 4260
tgtacatttt cacgctaata ctaatgtata attagaagat aatttttact catttttcgt 4320
tatcttcacg tcacccgaaac ctagaaccaa atgtcatttt cacgatatgt aaatagtgaa 4380
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gagaatggct tttatgaatc tagcaaactc tattcttggg gccggaataa ttactcagcc 4740
gttcgcgatc aaaaatgctg gtatattagg cgggctatta tcatacgtag ccctcggatt 4800
tatagttgat tggacgtaa gacttattgt cattaacttg actcttgctg gcaagagaac 4860
ataccagggt acggtcgaaac atgtaatggg taaaaaaggg aaattgctga ttctatttac 4920
aaacgggtta tttgcatttg gtggatgat tgg 4953

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<210> SEQ ID NO 76

<211> LENGTH: 606

<212> TYPE: PRT

<213> ORGANISM: Kluyveromyces dobzhanskii

<400> SEQUENCE: 76

```

Met His Thr Ile Ser Pro Glu Ser Asp Ser Leu Lys Ser Ser Lys Ser
1           5           10           15
Asn Thr Asn Ser His Glu Tyr Met Ser Ala Thr Thr Asp Tyr Asn Asn
20          25          30
Gly Gly Ala Ser Ala Ala Pro Leu Ser Ala Lys Arg Lys Glu Thr Gly
35          40          45
Asn Ser Tyr Glu Leu Gln Asp His Ala Ala Ser Ala Ser Ala Ser Ala
50          55          60
Ser Ala Ser Ala Ser Ala Ser Ala Ser Ala Ser Ala Leu Ala Phe Asp
65          70          75          80
Tyr Asp Pro Glu Asp Asp Asp Val Ser Ile Met Met Gln Asp Glu Glu
85          90          95
Val Pro Asp Leu Thr Trp Lys His Val Ser Asn Tyr Ala Ile Thr Arg
100         105         110
Phe Thr Ser Leu Ala His Ile His Pro Val Ser Met Gln Asn Ile Asn
115         120         125
Pro Phe Pro Glu Leu Arg Lys Met Thr Leu His Asn Trp Asn Tyr Phe
130         135         140

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Phe Met Gly Tyr Gly Ala Trp Leu Cys Ala Ser Trp Ala Phe Phe Ala
145 150 155 160
Val Ser Val Ser Thr Ala Pro Leu Ala Glu Leu Tyr Asp Lys Gln Thr
165 170 175
Lys Asp Ile Ser Trp Gly Leu Ser Leu Val Leu Phe Val Arg Ser Ala
180 185 190
Gly Ala Ile Ile Phe Gly Ile Trp Thr Asp Asn Tyr Ser Arg Lys Trp
195 200 205
Pro Tyr Ile Thr Cys Leu Gly Leu Phe Leu Ile Cys Gln Leu Cys Thr
210 215 220
Pro Phe Ala Thr Asn Tyr Thr Gln Phe Leu Gly Val Arg Trp Ile Ser
225 230 235 240
Gly Val Ala Met Gly Gly Ile Tyr Ala Cys Ala Ser Ala Thr Ala Ile
245 250 255
Glu Asp Ala Pro Val Lys Ala Arg Ser Phe Leu Ser Gly Leu Phe Phe
260 265 270
Thr Ala Tyr Ala Met Gly Phe Ile Phe Ala Thr Val Phe Tyr Arg Ala
275 280 285
Phe Leu Asn Ile Asn Gly Lys Asp Tyr Trp Lys Ile Gln Phe Trp Phe
290 295 300
Ser Ile Trp Leu Pro Ala Ala Leu Ile Leu Trp Arg Leu Val Trp Pro
305 310 315 320
Glu Thr Arg Tyr Phe Thr Lys Val Leu Lys Ala Arg Gln Leu Met Leu
325 330 335
Asp Asp Ala Ile Ala Ala Asn Gly Gly Gln Pro Leu Pro Lys Lys Thr
340 345 350
Phe Gln Glu Lys Val Thr Asn Val Lys Lys Ile Ile Ser Thr Tyr Trp
355 360 365
Leu Leu Phe Thr Tyr Leu Ile Leu Leu Leu Val Gly Pro Asn Tyr Leu
370 375 380
Thr His Ala Ser Met Asp Leu Phe Pro Thr Met Leu Arg Ala Gln Leu
385 390 395 400
Lys Phe Ser Glu Asp Ala Val Thr Val Ala Ile Val Val Val Cys Ile
405 410 415
Gly Ala Ile Cys Gly Gly Met Phe Phe Gly Gln Leu Met Glu Ile Thr
420 425 430
Gly Arg Arg Leu Gly Leu Leu Ile Ala Leu Val Met Ala Gly Cys Phe
435 440 445
Thr Tyr Pro Ala Phe Met Leu Asn Thr Gly Pro Ala Val Leu Gly Gly
450 455 460
Gly Phe Phe Leu Trp Phe Ser Val Ile Gly Val Trp Gly Val Ile Pro
465 470 475 480
Ile His Leu Ser Glu Leu Ser Pro Pro Glu Ala Arg Ala Leu Val Ser
485 490 495
Gly Leu Ala Tyr Gln Leu Gly Asn Leu Ala Ser Ala Ala Ser Val Val
500 505 510
Ile Glu Ser Asp Leu Ala Asp Leu Tyr Pro Ile Ala Trp Asn Ala Ala
515 520 525
Gly Glu Val Thr Asn Lys Asn Tyr Ser Lys Val Met Ala Ile Leu Thr
530 535 540
Gly Ser Val Val Ile Phe Thr Phe Val Leu Val Leu Val Gly His Glu
545 550 555 560

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caagttgaga gtaagagatc tgttcataca catcatagtg tgectatgaa ctcatcagat	1860
tcacaatcaa atgcgtcaga tcttgaggaa ttggcgccat ttccttctgt tgttgatgaa	1920
tcagaattag acgaatccga actagaggag atacaaaacg aagatgtctc tatcatttac	1980
aaagacgacg aagagaaacc agatttgagc tgggcatcaa tctgcagata cgctggtact	2040
cgtattactt ctttattoca aatccacaag gtgtctatgg ataacattaa tccattccct	2100
gaacttagaa agatgacatt gtccaattgg aactacttct tcatgggcta tttggcttgg	2160
ttgtgtgccc cctgggcctt cttcgctgtt tctgtateta cagcaccatt agcaaagctc	2220
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gctggggcta ttatctttgg actctggact gataactact ccagaaagtg gccatacatt	2340
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caattccttag gcgtaagatg gatatcaggt atcgcaatgg gtggtatata cggttgtgct	2460
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acaggtcgta gacttggact gttaattgct ttgactatgg ctggttgctt tacctaccct	3060
gctttcatgt tgaaaacatc ttcgcgctc ttaggtgcag gctttatgct ctttttctca	3120
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ccagcttact atccttcttg aaaatatgca ctctatatct tttagttctt aattgcaaca	3600
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ccatggagac atcaagatt gaaaatctat ggaaagatat ggacggtagc aacaagaata	3780
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gcttcagtga aaaaatcata aggaaaagtt gtaaatatta ttggtagtat tcgtttggtg	3900
aagtagaggg ggtaattttt cccctttatt ttgttcatac attcttaaat tgctttgcct	3960
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cgttttatta cctttgatca catttccacg ccatttcgca ttctcaccct cataagtc	4080
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ttttcacgct aataactaatg tataattaga agataatfff tactcatttt tcggtatcct 4320
cacgtcaccc gaacctagaa ccaaatgtca ttttcacgat atgtaaatag tgaatataggc 4380
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gaatctaaca gatatacatg ttccgataat gtctgagtta ggtgagtatt ctaaattaga 4500
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tgattggaag ttaagactta ttgtcattaa cttgactcct gctggcaaga gaacatacca 4860
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<210> SEQ ID NO 78

<211> LENGTH: 604

<212> TYPE: PRT

<213> ORGANISM: Kluyveromyces marxianus

<400> SEQUENCE: 78

```

Met Ser Glu Gly Asn Glu Glu Lys Gly Gln Gly Gln Gly Gln Glu
1          5          10          15
Asp His Gly His Phe Pro Glu Gly Arg Thr Phe Leu His Gln Val Glu
20          25          30
Ser Lys Arg Ser Val His Thr His His Ser Val Pro Met Asn Ser Ser
35          40          45
Asp Ser Gln Ser Asn Ala Ser Asp Pro Glu Glu Leu Ala Pro Phe Pro
50          55          60
Ser Val Val Asp Glu Ser Glu Leu Asp Glu Ser Glu Leu Glu Glu Ile
65          70          75          80
Gln Asn Glu Asp Val Ser Ile Ile Tyr Lys Asp Asp Glu Glu Lys Pro
85          90          95
Asp Leu Ser Trp Ala Ser Ile Cys Arg Tyr Ala Gly Thr Arg Ile Thr
100         105         110
Ser Leu Phe Gln Ile His Lys Val Ser Met Asp Asn Ile Asn Pro Phe
115         120         125
Pro Glu Leu Arg Lys Met Thr Leu Ser Asn Trp Asn Tyr Phe Phe Met
130         135         140
Gly Tyr Leu Ala Trp Leu Cys Ala Ala Trp Ala Phe Phe Ala Val Ser
145         150         155         160
Val Ser Thr Ala Pro Leu Ala Lys Leu Tyr Asp Lys Pro Thr Lys Asp
165         170         175
Ile Ser Trp Gly Leu Ser Leu Val Leu Phe Val Arg Ser Ala Gly Ala
180         185         190
Ile Ile Phe Gly Leu Trp Thr Asp Asn Tyr Ser Arg Lys Trp Pro Tyr
195         200         205
Ile Thr Cys Leu Gly Leu Phe Leu Ile Cys Gln Leu Cys Thr Pro Trp
210         215         220
Ala Lys Thr Tyr Thr Gln Phe Leu Gly Val Arg Trp Ile Ser Gly Ile

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225		230		235		240
Ala Met Gly Gly Ile Tyr Gly Cys Ala Ser Ala Thr Ala Ile Glu Asp						
		245		250		255
Ala Pro Val Lys Ala Arg Ser Ile Leu Ser Gly Leu Phe Phe Ser Ala		260		265		270
Tyr Ala Met Gly Phe Ile Phe Ala Ile Ile Phe Tyr Arg Ala Phe Leu		275		280		285
His Val Asn Gly Glu Asn Tyr Trp Lys Val Gln Phe Trp Phe Ser Ile		290		295		300
Phe Leu Pro Ala Leu Leu Met Leu Trp Arg Leu Val Trp Pro Glu Thr		305		310		315
				315		320
Lys Tyr Phe Thr Lys Val Leu Lys Ala Arg Glu Leu Met Lys Gln Asp		325		330		335
Ala Ile Ala Ser Asn Gly Gly Asn Pro Ile Pro Lys Thr Thr Phe Lys		340		345		350
Glu Lys Met Ala Asn Thr Lys Arg Ile Ile Cys Lys Tyr Trp Leu Leu		355		360		365
Phe Gly Tyr Leu Val Leu Leu Leu Val Gly Pro Asn Tyr Leu Thr His		370		375		380
Gly Ser Gln Asp Leu Phe Pro Thr Met Ile Arg Ser Gln Leu Asn Phe		385		390		395
				395		400
Ser Glu Asp Ala Val Thr Val Ala Ile Val Val Val Asn Leu Gly Gly		405		410		415
Ile Cys Gly Gly Leu Phe Phe Gly Gln Leu Met Glu Val Thr Gly Arg		420		425		430
Arg Leu Gly Leu Leu Ile Ala Leu Thr Met Ala Gly Cys Phe Thr Tyr		435		440		445
Pro Ala Phe Met Leu Lys Thr Ser Ser Ala Val Leu Gly Ala Gly Phe		450		455		460
Met Leu Phe Phe Ser Val Phe Gly Val Trp Gly Val Ile Pro Ile His		465		470		475
				475		480
Leu Ser Glu Leu Ser Pro Pro Glu Ala Arg Ala Leu Val Ala Gly Leu		485		490		495
Ala Tyr Gln Leu Gly Asn Leu Ala Ser Ala Ala Ser Val Val Ile Glu		500		505		510
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<210> SEQ ID NO 79

<211> LENGTH: 4707

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Yarrowia lipolytica.

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<400> SEQUENCE: 79

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 <211> LENGTH: 7332
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 <213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 80

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<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Aspergillus nidulans

<400> SEQUENCE: 81

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<210> SEQ ID NO 82

<211> LENGTH: 4983

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Saccharomyces bayanus

<400> SEQUENCE: 82

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<210> SEQ ID NO 83

<211> LENGTH: 615

<212> TYPE: PRT

<213> ORGANISM: *Saccharomyces bayanus*

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 35 40 45
 Asn Pro Asp His Glu Lys Leu Tyr His Asn Pro Ser Leu Pro Val Thr
 50 55 60
 Ser Ile Gln Asp Thr Arg Asp Asp Glu Leu Leu Glu Arg Val Tyr Ser
 65 70 75 80
 Val Asp Gln Glu Val Glu Tyr Glu Glu Asp Glu Ala Asp Lys Pro Asn
 85 90 95
 Leu Ser Leu Ala Ser Ile Lys Asn Tyr Ala Leu Thr Arg Phe Thr Ser
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 Asp Thr Tyr Gln Lys Phe Leu Gly Val Arg Trp Ile Thr Gly Ile Ala
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 245 250 255
 Pro Val Lys Ala Arg Ser Phe Leu Ser Gly Leu Phe Phe Ser Ala Tyr
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 Ala Met Gly Phe Ile Phe Ala Ile Ile Phe Tyr Arg Ala Phe Gly Tyr
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 290 295 300
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 Phe Thr Lys Val Leu Lys Ala Arg Arg Leu Ile Leu Ser Asp Ala Val
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 Lys Ala Asn Gly Gly Glu Pro Leu Pro Lys Ala Asn Phe Lys Gln Lys
 340 345 350
 Met Val Ser Val Lys Lys Thr Val Gln Lys Tyr Trp Leu Leu Phe Ala
 355 360 365
 Tyr Leu Val Ile Met Leu Val Gly Pro Asn Tyr Leu Thr His Ala Ser
 370 375 380
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 385 390 395 400
 Asp Ala Val Thr Val Ile Val Val Val Thr Asn Ile Gly Ala Ile Cys
 405 410 415

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 450 455 460
 Tyr Phe Cys Val Phe Gly Val Trp Gly Ile Leu Pro Ile His Leu Ala
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 485 490 495
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 565 570 575
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<210> SEQ ID NO 84

<211> LENGTH: 5192

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Kluyveromyces lactis

<400> SEQUENCE: 84

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gttgacattg cgaagagcga caaagathtt gttatcggct ttattgctca aagagacatg      360
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<210> SEQ ID NO 85

<211> LENGTH: 4825

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae and Kluyveromyces lactis

<400> SEQUENCE: 85

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<210> SEQ ID NO 86
 <211> LENGTH: 5157
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Sequence put together from multiple organisms:
 Saccharomyces cerevisiae and Kluyveromyces lactis

<400> SEQUENCE: 86

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<210> SEQ ID NO 87

<211> LENGTH: 5600

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Sequence put together from multiple organisms:
Saccharomyces cerevisiae, *Kluyveromyces lactis*, and *Aspergillus nidulans*

<400> SEQUENCE: 87

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<210> SEQ ID NO 88

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The invention claimed is:

1. A genetically modified yeast comprising:

a heterologous gene encoding monocarboxylic/monocarboxylate transporter polypeptide having monocarboxylic/monocarboxylate transporter activity, wherein the said monocarboxylic/monocarboxylate transporter polypeptide comprises an amino acid sequence having at least 90% sequence identity to any one of the following amino acid sequences: SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 76, or SEQ ID NO: 78, and

one or more heterologous genes encoding a lactate dehydrogenase polypeptide having lactate dehydrogenase activity, wherein the said lactate dehydrogenase poly-

peptide comprises an amino acid sequence having at least 90% sequence identity to any one of the following amino acid sequences: SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, or SEQ ID NO: 74, and

wherein the yeast is capable of consuming lactate and producing ethanol at a titer greater than 80 g/L when the yeast is present in a fermentation medium comprising lactate and hexose.

2. The yeast of claim 1, wherein the yeast has a L-lactate consumption rate of at least 0.015 gL⁻¹h⁻¹ when the concentration of hexose in the fermentation medium is 5 g/L or greater at a sampling time of 48 h, as measured according to the Evaluation Protocol for Lactate Consumption.

3. The yeast of claim 1, wherein the yeast has a D-lactate consumption rate of at least $0.015 \text{ gL}^{-1}\text{h}^{-1}$ when the concentration of hexose in the fermentation medium is 5 g/L or greater at a sampling time of 48 h, as measured according to the Evaluation Protocol for Lactate Consumption. 5

4. The yeast of claim 1, wherein the D-, L-, and/or total lactate consumption rate of the yeast is greater than a yeast without a heterologous gene encoding a monocarboxylic/monocarboxylate transporter.

5. The yeast of claim 1, wherein the yeast is capable of consuming both D-lactate and L-lactate when the yeast is present in a fermentation medium comprising lactate and hexose. 10

6. The yeast of claim 1, wherein the yeast is genetically modified from a host yeast of the species *Saccharomyces cerevisiae*. 15

7. A process for producing ethanol comprising fermenting a substrate with the yeast of claim 1.

8. The process of claim 7, having a volumetric oxygen uptake rate (OUR) of at least $0.5 \text{ mmol O}_2/(\text{L}\cdot\text{h})$. 20

9. The process of claim 7, wherein the ethanol titer at the end of fermentation is at least 100 g/liter.

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